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Cracking the Bioactive Code of *Rehmannia glutinosa*: Analysis and Functions of Active Components

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Abstract *Rehmannia glutinosa*, a cornerstone of traditional Chinese medicine, is renowned for its diverse pharmacological properties attributed to its bioactive components. This research delves into the intricate bioactive code of *R. glutinosa*, focusing on the analysis and functions of its active components. Notably, acteoside, a prominent bioactive compound, exhibits significant health benefits, yet its biosynthetic pathway remains partially understood. Recent studies have identified a novel tyrosine decarboxylase (RgTyDC2) from the *R. glutinosa* transcriptome, which plays a crucial role in the biosynthesis of acteoside by converting tyrosine and dopa into tyramine and dopamine, respectively, with a higher catalytic efficiency towards tyrosine. Additionally, the dynamic accumulation of various glycosides and saccharides, including catalpol, acteoside, and ajugol, has been investigated in both the roots and leaves of *R. glutinosa*. This research highlights the potential of utilizing the often-discarded leaves, which contain significant amounts of these bioactive compounds, thereby enhancing the resource value of the plant. This research synthesizes current findings to provide a comprehensive understanding of the bioactive components of *R. glutinosa*, their biosynthesis, and their potential applications in medicine.

Keywords *Rehmannia glutinosa*; Acteoside; Tyrosine decarboxylase; Glycosides; Pharmacological activities

1 Introduction

Rehmannia glutinosa, a perennial herb belonging to the Scrophulariaceae family, has been a cornerstone in traditional Chinese medicine (TCM) for centuries. Historically, it has been utilized for its purported benefits in treating a variety of ailments, including anemia, diabetes, and fever. The roots of *R. glutinosa*, often referred to as "Di Huang," are particularly valued and have been processed through methods such as steaming and drying to enhance their medicinal properties (Kwon et al., 2019).

In TCM, *R. glutinosa* is considered a vital herb due to its wide range of pharmacological activities. It is frequently used in formulations aimed at nourishing the blood, tonifying the kidneys, and replenishing vital essence. The herb's significance is further underscored by its inclusion in numerous classical TCM prescriptions. Modern research has identified several bioactive compounds in *R. glutinosa*, such as catalpol and acteoside, which contribute to its therapeutic effects (Zhi et al., 2018; Li et al., 2020; Cai et al., 2024).

The primary objective of this research is to provide a comprehensive analysis of the active components of *Rehmannia glutinosa* and their functions. By examining recent studies, elucidate the molecular mechanisms underlying the biosynthesis of these bioactive compounds and their pharmacological activities, will also explore the potential applications of these findings in enhancing the medicinal value of *R. glutinosa* and developing new therapeutic agents.

2 Botanical Characteristics of *Rehmannia glutinosa*

2.1 Taxonomy and morphology

Rehmannia glutinosa, commonly known as Chinese foxglove, belongs to the family Scrophulariaceae. It is a perennial herbaceous plant that has been widely used in traditional Chinese medicine for its medicinal properties, particularly its roots (Zhang et al., 2021; Qin et al., 2022). The plant is characterized by its rosette of basal leaves and a flowering stem that can reach up to 30~50 cm in height. The leaves are ovate to lanceolate, with a serrated margin, and are covered with fine hairs. The flowers are tubular, typically purple or yellow, and are arranged in a raceme. The fruit is a capsule that contains numerous small seeds (Li et al., 2018; Xia et al., 2021a).

2.2 Geographical distribution and cultivation practices

Rehmannia glutinosa is native to China and has been cultivated for centuries in various regions, including Henan, Shandong, and Hebei provinces. It is also grown in Korea, Japan, and northern Vietnam (Xia et al., 2021a; Qin et al., 2022). The plant thrives in well-drained, fertile soils with a pH range of 5.5 to 7.5 and requires a temperate climate with adequate rainfall. The cultivation of *R. glutinosa* involves both sexual reproduction through seeds and asexual reproduction through rootstock. However, the latter is more common due to its higher efficiency and reliability in producing uniform plants (Kim et al., 2020).

In Henan Province, which is a major cultivation area, *R. glutinosa* is typically planted in spring and harvested in late autumn. The cultivation practices include soil preparation, planting, irrigation, fertilization, and pest management. The soil is usually plowed and enriched with organic matter before planting. The rootstocks are planted at a depth of 5~10 cm and spaced 20~30 cm apart. Regular irrigation is essential, especially during dry periods, to ensure optimal growth and development (Kim et al., 2020; Zhang et al., 2021).

One of the significant challenges in the cultivation of *R. glutinosa* is the prevalence of viral diseases, which can severely affect the yield and quality of the roots. Studies have identified several viruses that infect *R. glutinosa*, including Rehmannia mosaic virus (ReMV), cucurbit chlorotic yellows virus (CCYV), and tobacco mild green mosaic virus (TMGMV) (Zhang et al., 2021; Qin et al., 2022). These viral infections are often exacerbated by the asexual reproduction method, which can facilitate the spread of viruses through the rootstock. To mitigate this issue, researchers have developed in vitro tissue culture methods to produce virus-free seedlings and rootstocks, which have shown promising results in improving the productivity and quality of *R. glutinosa* (Kim et al., 2020).

In addition to traditional cultivation methods, modern biotechnological approaches are being employed to enhance the production and quality of *R. glutinosa*. For instance, the use of scanning electron microscopy (SEM) and quantitative taxonomy has enabled the identification and classification of different germplasms, which can be used for breeding superior cultivars (Li et al., 2018). Furthermore, the complete chloroplast genome sequencing of *R. glutinosa* cultivars has provided valuable insights into the genetic diversity and evolutionary history of the species, which can aid in conservation and breeding programs (Xia et al., 2021b).

Overall, the cultivation of *Rehmannia glutinosa* involves a combination of traditional agricultural practices and modern biotechnological techniques to ensure the production of high-quality medicinal roots. The ongoing research and development efforts aim to address the challenges associated with viral diseases and improve the overall productivity and sustainability of *R. glutinosa* cultivation (Li et al., 2018; Kim et al., 2020; Xia et al., 2021b; Zhang et al., 2021; Qin et al., 2022).

3 Chemical Composition of *Rehmannia glutinosa*

3.1 Overview of phytochemical constituents

Rehmannia glutinosa, a traditional Chinese medicinal herb, is known for its diverse array of bioactive compounds. The primary phytochemicals identified in *R. glutinosa* include iridoid glycosides, phenethyl alcohol glycosides, polysaccharides, and various minor constituents. These compounds contribute to the plant's therapeutic properties, such as anti-inflammatory, antioxidant, and hypoglycemic effects (Dai et al., 2018; Jeong et al., 2020; Piątczak et al., 2020).

3.2 Major bioactive components

3.2.1 Iridoid glycosides

Iridoid glycosides are among the most significant bioactive components in *R. glutinosa*. Key iridoid glycosides include aucubin, catalpol, and geniposide. These compounds have been extensively studied for their pharmacokinetic properties and biological activities. For instance, a validated method for the simultaneous determination of aucubin, catalpol, and geniposide in rat biological samples has shown high precision and accuracy, making it suitable for pharmacokinetic studies (Jeong et al., 2020). Additionally, the biosynthesis of iridoid glycosides in *R. glutinosa* involves several key genes, such as DXS, DXR, and GPPS, which are upregulated under specific conditions, enhancing the accumulation of these compounds (Dong et al., 2022) (Figure 1). New iridoid glycosides, such as rehmaglutosides L-O, have also been identified, further expanding the understanding of this class of compounds (Liu et al., 2023).

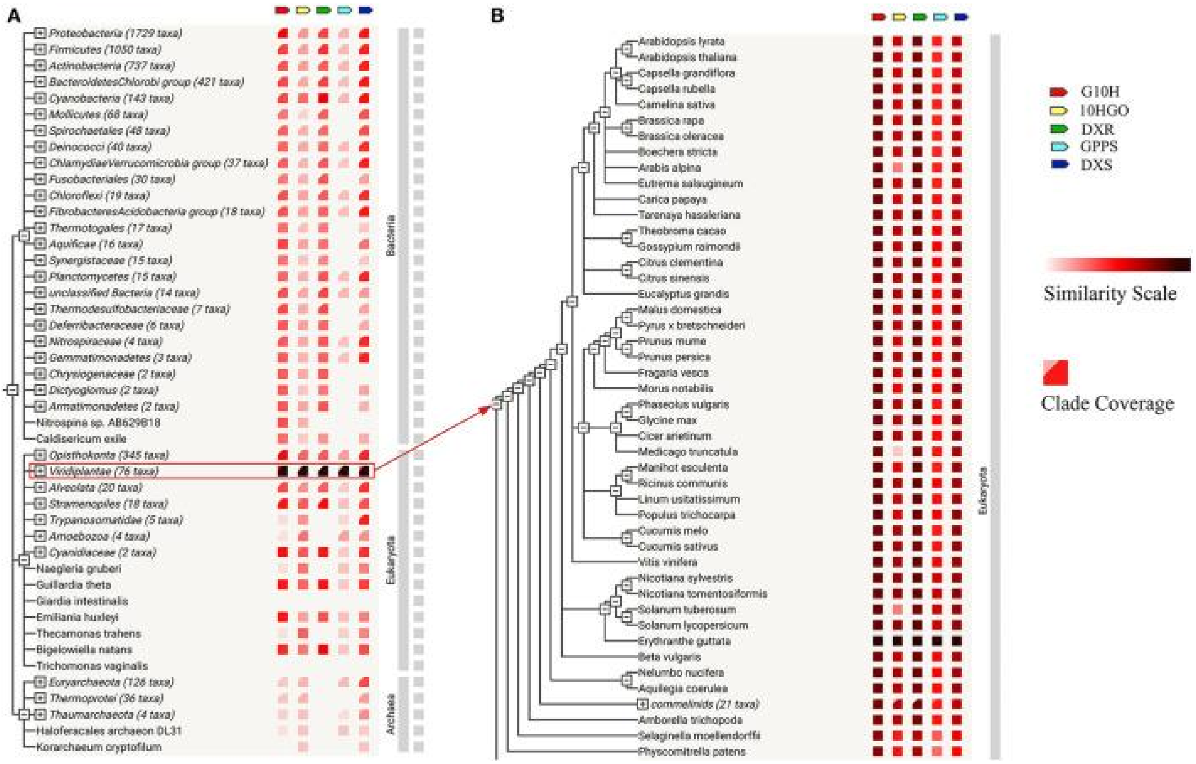

Figure 1 Co-occurrence on iridoid glycoside synthetase genes of *R. glutinosa* (Adopted from Dong et al., 2022)

Image caption: (A): Gene co-occurrence of DXS, DXR, GPPS, G10H, and 10HGO in organism; (B): The co-occurrence of DXS, DXR, GPPS, G10H, and 10HGO in plants; Similarity scale: The color indicates the similarity of gene and its homology in the given STRING. Clade coverage: Two distinc tcolors, respectively, denote the lowest and highest similarity observed within the clade (Adopted from Dong et al., 2022)

3.2.2 Phenethyl alcohol glycosides

Phenethyl alcohol glycosides, including verbascoside, are another important group of bioactive compounds in *R. glutinosa*. These glycosides exhibit significant antimicrobial and cytotoxic activities. For example, verbascoside has been found in high concentrations in shoots cultured under specific light conditions, demonstrating its potential for enhanced production through controlled cultivation (Piątczak et al., 2020). The presence of phenethyl alcohol glycosides contributes to the plant's therapeutic effects, such as anti-inflammatory and antioxidant activities (Zhang et al., 2019).

3.2.3 Polysaccharides

Polysaccharides from *R. glutinosa* have garnered attention for their immunomodulatory properties. *Rehmannia glutinosa* polysaccharide (RGP) has been shown to activate dendritic cells in the mediastinal lymph node, enhancing immune responses. This activation is characterized by increased expression of co-stimulatory molecules and pro-inflammatory cytokines, indicating the potential of RGP as a mucosal adjuvant (Kwak et al., 2018). Additionally, polysaccharides in *R. glutinosa* leaves and roots have been quantified, revealing significant concentrations that contribute to the plant's overall bioactivity (Xu et al., 2019).

3.2.4 Other minor constituents

In addition to the major bioactive components, *R. glutinosa* contains various minor constituents that contribute to its medicinal properties. These include new compounds such as rehmannia A and ionone rehmannias B-C, which have shown moderate activity in suppressing α-glucosidase, a key enzyme involved in carbohydrate metabolism (Li et al., 2023). The identification of these minor constituents provides new insights into the hypoglycemic effects of *R. glutinosa* and its potential for developing α-glucosidase inhibitor drugs. Furthermore, the antioxidant activity of *R. glutinosa* has been linked to its diverse phytochemical profile, with certain samples exhibiting high levels of catalpol, rehmaionoside A, and rehmannioside D, which contribute to its strong antioxidant properties (Liu et al., 2020).

In summary, the chemical composition of *Rehmannia glutinosa* is complex and diverse, with iridoid glycosides, phenethyl alcohol glycosides, polysaccharides, and various minor constituents playing crucial roles in its bioactivity. These compounds collectively contribute to the plant's therapeutic potential, making it a valuable resource in traditional and modern medicine.

4 Analytical Techniques for Component Identification

4.1 Chromatographic methods

4.1.1 High-performance liquid chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is a widely used technique for the separation, identification, and quantification of components in complex mixtures. In the context of *Rehmannia glutinosa*, HPLC has been employed to analyze various bioactive compounds, ensuring the quality and consistency of the herbal material. The technique's high resolution and sensitivity make it ideal for detecting minor components and impurities, which is crucial for both research and clinical applications.

4.1.2 Gas chromatography-mass spectrometry (GC-MS)

Gas Chromatography-Mass spectrometry (GC-MS) combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. This method is particularly useful for analyzing volatile and semi-volatile compounds. In studies involving *Rehmannia glutinosa*, GC-MS has been used to profile the metabolic components and to understand the pharmacokinetics of the herb in biological systems. For instance, the UPLC-Q-TOF/MS technique, which is closely related to GC-MS, has been applied to analyze the metabolic profiles of bioactive components in *Rehmannia glutinosa*, providing insights into their absorption and metabolism in both normal and CKD rats (Tao et al., 2018).

4.2 Spectroscopic methods

4.2.1 Nuclear magnetic resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique used to determine the structure of organic compounds. NMR provides detailed information about the molecular structure, dynamics, reaction state, and chemical environment of molecules. In the study of *Rehmannia glutinosa*, NMR can be used to elucidate the structures of complex polysaccharides and other bioactive molecules, contributing to a better understanding of their functional properties and potential therapeutic effects.

4.2.2 Infrared spectroscopy (IR)

Infrared Spectroscopy (IR) is another essential tool for the identification of chemical compounds based on their vibrational transitions. Fourier Transform Infrared Spectroscopy (FTIR) has been specifically utilized to analyze the chemical composition of *Rehmannia glutinosa* and its extracts. FTIR, combined with second derivative spectrum and thermal analysis, has proven effective in distinguishing subtle differences in the chemical components of various types of *Rehmannia glutinosa*, thereby aiding in quality control and standardization of the herbal material (Zhang et al., 2022).

4.3 Other analytical approaches

In addition to chromatographic and spectroscopic methods, other advanced analytical techniques have been employed to study the bioactive components of *Rehmannia glutinosa*. For example, Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) combined with machine learning has been used to visualize the spatial distribution of oligosaccharides in processed *Rehmannia glutinosa*. This approach not only helps in understanding the metabolic changes during processing but also aids in the standardization and modernization of medicinal plant processing (Li et al., 2023). Another innovative method is Internal Extractive Electrospray Ionization Mass Spectrometry (iEESI-MS), which, when combined with statistical techniques, allows for high-throughput molecular differentiation of *Rehmannia glutinosa* samples from different origins, providing a rapid and efficient means of quality assessment (Xu et al., 2023).

These analytical techniques collectively contribute to a comprehensive understanding of the bioactive components in *Rehmannia glutinosa*, facilitating their identification, quantification, and functional analysis. This integrated approach is essential for advancing the therapeutic applications and ensuring the quality and efficacy of this traditional medicinal herb.

5 Pharmacological Activities of Bioactive Components

5.1 Iridoid glycosides

5.1.1 Anti-inflammatory effects

Iridoid glycosides, such as catalpol, are prominent bioactive components in *Rehmannia glutinosa*. These compounds have demonstrated significant anti-inflammatory properties. For instance, catalpol has been shown to inhibit the production of nitric oxide (NO) and suppress the expression of inducible nitric oxide synthase (iNOS), which are key mediators of inflammation (Rahmat et al., 2022). Additionally, catalpol's ability to modulate inflammatory pathways suggests its potential as a therapeutic agent for inflammatory diseases.

5.1.2 Neuroprotective effects

Catalpol also exhibits neuroprotective effects, making it a promising candidate for the treatment of neurodegenerative diseases. Studies have shown that catalpol can protect neuronal cells from oxidative stress and apoptosis, which are critical factors in the progression of neurodegenerative conditions (Ge et al., 2023). The neuroprotective mechanisms of catalpol involve the upregulation of neurotrophic factors and the inhibition of pro-apoptotic pathways, thereby promoting neuronal survival and function.

5.2 Phenethyl alcohol glycosides

5.2.1 Antioxidant properties

Phenethyl alcohol glycosides, such as acteoside, are known for their potent antioxidant properties. Acteoside has been found to scavenge free radicals and reduce oxidative stress, which is beneficial in preventing cellular damage and aging (Liu et al., 2020). The antioxidant activity of acteoside is attributed to its ability to donate hydrogen atoms and stabilize free radicals, thereby protecting cells from oxidative damage.

5.2.2 Hepatoprotective effects

Acteoside also exhibits hepatoprotective effects, which are crucial for liver health. Research has shown that acteoside can protect liver cells from damage induced by toxins and oxidative stress (Li et al., 2022). The hepatoprotective mechanisms of acteoside include the enhancement of antioxidant defenses and the inhibition of inflammatory responses in the liver, which help to maintain liver function and prevent liver diseases.

5.3 Polysaccharides

5.3.1 Immunomodulatory effects

Polysaccharides from *Rehmannia glutinosa* have been shown to possess significant immunomodulatory effects. These polysaccharides can enhance the immune response by stimulating the proliferation and activation of immune cells, such as macrophages and lymphocytes (Chen et al., 2022). The immunomodulatory properties of these polysaccharides make them valuable for boosting the immune system and improving resistance to infections and diseases.

5.3.2 Antitumor activities

In addition to their immunomodulatory effects, polysaccharides from *Rehmannia glutinosa* also exhibit antitumor activities. Studies have demonstrated that these polysaccharides can inhibit the growth of tumor cells and induce apoptosis, thereby reducing tumor progression (Chen et al., 2022). The antitumor mechanisms involve the activation of immune responses against tumor cells and the direct induction of tumor cell death.

5.4 Synergistic effects of combined components

The combined use of different bioactive components from *Rehmannia glutinosa* can result in synergistic effects, enhancing their overall pharmacological activities. For example, the combination of catalpol, acteoside, and echinacoside has been shown to enhance bone formation and prevent bone loss in diabetic rats by regulating the IGF-1/PI3K/mTOR signaling pathways (Gong et al., 2019). This synergistic interaction highlights the potential of using multiple components from *Rehmannia glutinosa* to achieve more effective therapeutic outcomes. Additionally, the combined antioxidant and anti-inflammatory properties of these components can provide comprehensive protection against oxidative stress and inflammation-related diseases (Liu et al., 2020; Rahmat et al., 2022).

In conclusion, the bioactive components of *Rehmannia glutinosa*, including iridoid glycosides, phenethyl alcohol glycosides, and polysaccharides, exhibit a wide range of pharmacological activities. These activities include anti-inflammatory, neuroprotective, antioxidant, hepatoprotective, immunomodulatory, and antitumor effects. The synergistic interactions between these components further enhance their therapeutic potential, making *Rehmannia glutinosa* a valuable medicinal plant for various health conditions.

6 Mechanisms of Action

6.1 Cellular and molecular targets

Rehmannia glutinosa, a traditional Chinese medicinal herb, contains several bioactive compounds such as acteoside, catalpol, and ferulic acid, which have been shown to target various cellular and molecular components. Acteoside, for instance, is synthesized through the action of tyrosine decarboxylase (RgTyDC2), which converts tyrosine into tyramine, a precursor in acteoside biosynthesis (Li et al., 2022). Similarly, ferulic acid biosynthesis involves the enzyme caffeic acid O-methyltransferase (RgCOMT), which catalyzes the methylation of caffeic acid (Yang et al., 2023). These enzymes are crucial for the production of bioactive compounds that interact with cellular targets to exert their pharmacological effects.

6.2 Signal transduction pathways

The bioactive compounds in *Rehmannia glutinosa* influence various signal transduction pathways. For example, phenolic compounds produced via the phenylpropanoid pathway, such as those catalyzed by cinnamate 4-hydroxylase (RgC4H), play a significant role in the plant's response to oxidative stress by activating antioxidant systems (Yang et al., 2021). The overexpression of RgC4H enhances the plant's tolerance to oxidative stress by modulating the phenolics/phenylpropanoid pathway, which in turn activates molecular networks that mitigate the effects of drought, salinity, and hydrogen peroxide-induced stress (Yang et al., 2021). Additionally, the suppression of pro-inflammatory cytokine IL-6 in lipopolysaccharide-stimulated macrophages by puffed *Rehmannia glutinosa* extracts indicates the involvement of anti-inflammatory pathways (Kwon et al., 2019).

6.3 Genetic and epigenetic regulation

The biosynthesis of bioactive compounds in *Rehmannia glutinosa* is tightly regulated at the genetic and epigenetic levels. The expression of genes involved in the phenylpropanoid pathway, such as phenylalanine ammonia-lyase (RgPAL), is crucial for the production of phenolic compounds (Yang et al., 2021). Overexpression of RgPAL genes has been shown to enhance phenolic production and release, which is associated with the development of replanting disease due to autotoxic harm (Yang et al., 2021). Furthermore, the differential expression of genes in radial striation (RS) and non-radial striation (nRS) tissues of *Rehmannia glutinosa* tuberous roots highlights the tissue-specific regulation of catalpol and acteoside biosynthesis (Zhi et al., 2018). Transcriptome analysis has identified key genes and transcription factors that are differentially expressed in these tissues, providing insights into the genetic regulation of bioactive compound accumulation (Zhi et al., 2018) (Figure 2).

In conclusion, the bioactive compounds of *Rehmannia glutinosa* target specific cellular and molecular components, modulate various signal transduction pathways, and are regulated by complex genetic and epigenetic mechanisms. Understanding these mechanisms is essential for harnessing the therapeutic potential of this medicinal herb.

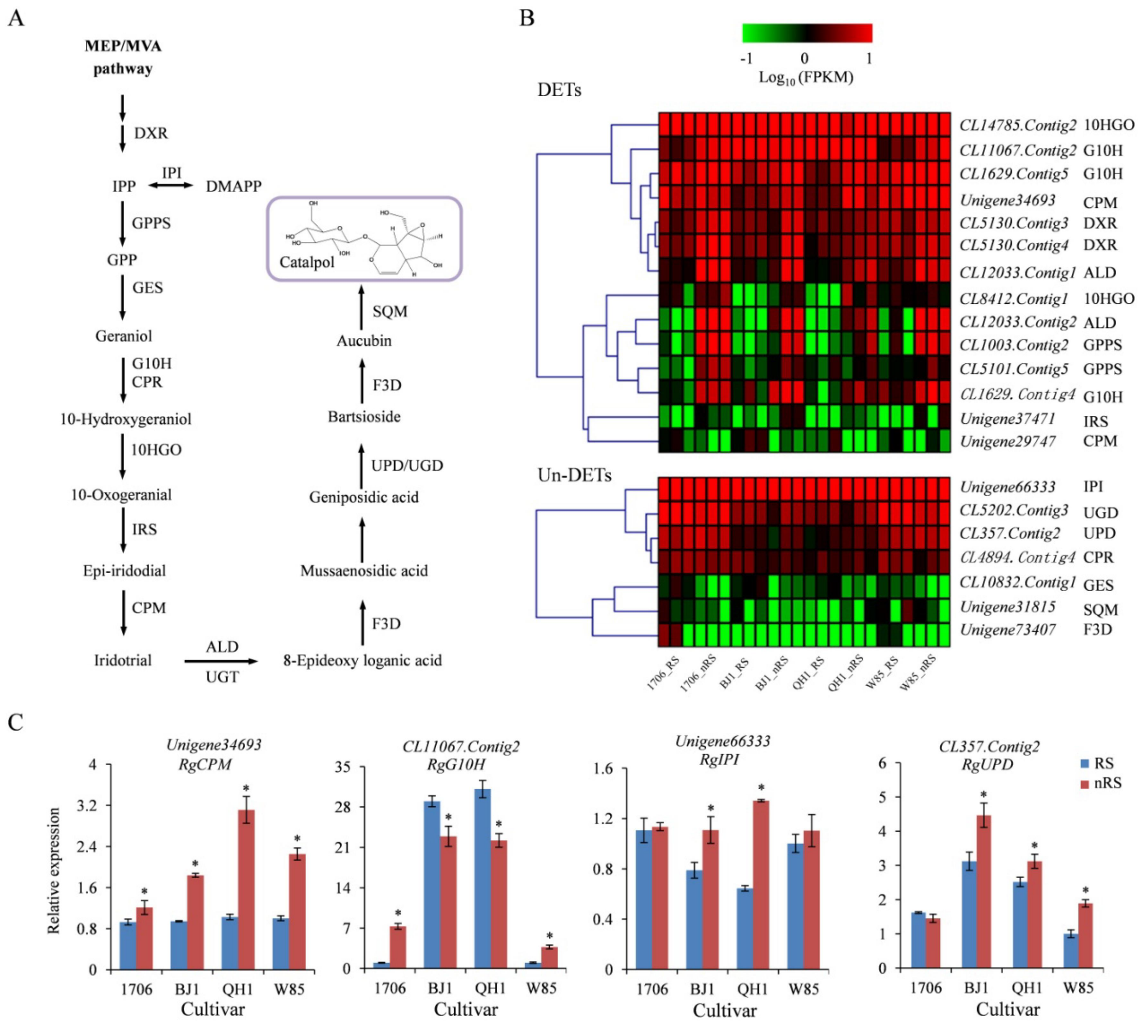

Figure 2 Expression patterns of catalpol biosynthetic unigenes between radial striations and non-radial striations of *R. glutinosa* (Adopted from Zhi et al., 2018)

Image caption: (A) Biosynthetic pathway of catalpol. (B) Heat map representing expression dynamics of unigenes involved in biosynthesis. The expression values (FPKM) for unigenes were log10 transformed and scaled across each row, and the heatmap was generated by MultiExperiment Viewer (MeV). (C) Relative expression of candidate genes in involved in catalpol biosynthesis. These genes expression levels were all determined by Real-time PCR (qRT-PCR). Vertical bars indicate the standard deviation of three biological replicates. Asterisks (*) indicate a significant difference at the $p<0.05$ level. Abbreviations: DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; GPPS, geranyl diphosphate synthase; IPI, isopentenyl-diphosphate Delta-isomerase; GES, geraniol synthase; G10H, Geraniol 10-hydroxylase; CPR, NADPH--cytochrome P450 reductase; 10HGO, 10-hydroxygeraniol dehydrogenase; IRS, iridoid synthase; CPM, Cytochrome P-450 monooxygenase; ALD, aldehyde dehydrogenase; UGT, Uridine diphosphate glycosyltransferase; F3D, flavanone 3-dioxygenase; UPD, uroporphyrinogen decarboxylase; UGD, UDP-glucuronic acid decarboxylase; SQM, squalene monooxygenase (Adopted from Zhi et al., 2018)

7 Clinical Applications and Therapeutic Potential

7.1 Current clinical use

7.1.1 Traditional formulations

Rehmannia glutinosa has been a cornerstone in traditional Chinese medicine (TCM) for centuries. It is often used in various formulations to treat a range of ailments, particularly those related to blood sugar regulation and immune function. Traditional formulations typically involve the use of dried or processed roots, which are believed to possess potent medicinal properties. For instance, the hypoglycemic effects of *R. glutinosa* have been well-documented, with studies showing its ability to lower blood glucose levels and improve lipid metabolism in diabetic models (Qin et al., 2018; Li et al., 2023). Additionally, its antioxidant properties have been highlighted, suggesting its potential in combating oxidative stress-related conditions (Liu et al., 2020).

7.1.2 Modern herbal preparations

In recent years, modern herbal preparations of *R. glutinosa* have been developed to enhance its therapeutic efficacy and ease of use. These preparations often involve the extraction and concentration of bioactive compounds such as acteoside, catalpol, and polysaccharides. For example, PEGylation nano-adjuvants based on *R. glutinosa* polysaccharides have been designed to improve drug-targeting effects and immunological functions, showing promising results in macrophage activation and cytokine production (Huang et al., 2019). Such advancements not only preserve the traditional benefits of *R. glutinosa* but also enhance its clinical applicability in modern medicine.

7.2 Potential therapeutic applications

7.2.1 Chronic diseases

R. glutinosa has shown significant potential in the management of chronic diseases, particularly diabetes and its complications. Studies have demonstrated that *R. glutinosa* and its combinations with other herbs can effectively lower fasting blood glucose levels, improve glucose tolerance, and regulate lipid profiles in diabetic models (Qin et al., 2018; Li et al., 2023). The identification of compounds that inhibit α-glucosidase further supports its role in diabetes management, providing a mechanistic understanding of its hypoglycemic effects (Qin et al., 2018). Additionally, the antioxidant properties of *R. glutinosa* suggest its potential in mitigating oxidative stress, a common factor in many chronic diseases (Qin et al., 2018; Li et al., 2023).

7.2.2 Age-related disorders

The bioactive components of *R. glutinosa*, such as acteoside and catalpol, have been associated with anti-aging properties. These compounds exhibit antioxidant and anti-inflammatory activities, which are crucial in combating age-related disorders. For instance, acteoside has been shown to protect against oxidative damage and improve cellular functions, which could be beneficial in conditions like Alzheimer's disease and other neurodegenerative disorders (Zhou et al., 2021; Li et al., 2022). Moreover, the enhancement of ferulic acid production through genetic engineering of *R. glutinosa* enzymes opens new avenues for its use in age-related therapeutic applications (Yang et al., 2023).

7.3 Safety and toxicology

The safety profile of *R. glutinosa* is generally considered favorable, with traditional use spanning centuries. However, modern studies have begun to scrutinize its safety and potential toxicological effects more rigorously. For instance, the development of PEGylation nano-adjuvants has highlighted the importance of optimizing preparation methods to ensure safety and efficacy (Huang et al., 2019). While no significant adverse effects have been reported in the studies reviewed, it is crucial to conduct comprehensive toxicological assessments to confirm the long-term safety of both traditional and modern preparations of *R. glutinosa*. Future research should focus on identifying any potential toxic compounds and establishing safe dosage ranges to maximize therapeutic benefits while minimizing risks.

In conclusion, *Rehmannia glutinosa* holds significant promise in both traditional and modern medical applications. Its bioactive components offer therapeutic potential for managing chronic diseases and age-related disorders, while ongoing research into its safety and efficacy will help to solidify its role in contemporary medicine.

8 Future Directions and Research Prospects

8.1 Gaps in current research

Despite significant advancements in understanding the bioactive components of *Rehmannia glutinosa*, several gaps remain. One major gap is the limited exploration of the chemical constituents in the non-medicinal parts of the plant, such as the stems and leaves. Recent studies have shown that these parts contain similar bioactive compounds as the roots, yet they are often discarded during harvesting (Xu et al., 2019). This indicates a potential for broader utilization of the plant, which has not been fully explored. Additionally, while the biosynthesis pathways of certain compounds like ferulic acid (FA) have been elucidated, the complete metabolic networks and regulatory mechanisms governing the production of other significant bioactive molecules remain unclear (Yang et al., 2019). More comprehensive studies are needed to map out these pathways and understand the interactions between different biosynthetic routes.

8.2 Emerging technologies in phytochemical research

The advent of advanced analytical techniques has revolutionized phytochemical research, providing new opportunities to delve deeper into the bioactive components of *Rehmannia glutinosa*. Technologies such as ultra-high-performance liquid chromatography (UHPLC) coupled with triple quadrupole tandem mass spectrometry (MS/MS) and high-performance liquid chromatography (HPLC) have enabled rapid and precise quantification of various glycosides and saccharides in the plant (Xu et al., 2019). These methods allow for a more detailed analysis of the dynamic accumulation of these compounds at different growth stages and in different plant parts. Furthermore, the use of synthetic biology and metabolic engineering, as demonstrated by the reconstitution of the FA biosynthetic pathway in Saccharomyces cerevisiae, opens new avenues for the production of plant-derived compounds in microbial hosts (Yang et al., 2019). This approach not only facilitates the study of complex biosynthetic pathways but also offers a scalable method for producing valuable bioactive compounds.

8.3 Prospective clinical trials and studies

To fully harness the therapeutic potential of *Rehmannia glutinosa*, it is imperative to conduct rigorous clinical trials and studies. While in vitro and in vivo studies have provided insights into the pharmacological effects of its bioactive components, clinical validation is essential to confirm their efficacy and safety in humans. Future research should focus on designing well-structured clinical trials to evaluate the therapeutic benefits of compounds such as catalpol, acteoside, and ferulic acid. Additionally, exploring the synergistic effects of these compounds could lead to the development of more effective multi-component herbal formulations. The identification and functional characterization of key enzymes involved in the biosynthesis of these compounds, such as the RgCOMT enzyme for FA production, also pave the way for genetic and metabolic engineering approaches to enhance the yield and consistency of bioactive components in *Rehmannia glutinosa* (Yang et al., 2019). These advancements could significantly impact the development of standardized and potent herbal medicines.

In conclusion, while substantial progress has been made in understanding the bioactive components of *Rehmannia glutinosa*, addressing the existing research gaps, leveraging emerging technologies, and conducting comprehensive clinical trials are crucial steps towards fully realizing the therapeutic potential of this valuable medicinal plant.

9 Conclusion Remarks

Rehmannia glutinosa, a prominent medicinal plant in traditional Chinese medicine, has been extensively studied for its bioactive components and their pharmacological activities. Key findings from recent research highlight the identification and functional characterization of several enzymes and compounds that contribute to the biosynthesis of these bioactive components.

Tyrosine Decarboxylase (RgTyDC2): A novel tyrosine decarboxylase was identified, which plays a crucial role in the biosynthesis of acteoside, a significant bioactive compound in *R. glutinosa*. RgTyDC2 catalyzes the conversion of tyrosine to tyramine and dopa to dopamine, with a higher efficiency towards tyrosine. The expression pattern of RgTyDC2 correlates with acteoside accumulation, suggesting its involvement in acteoside biosynthesis.

Caffeic Acid O-methyltransferase (RgCOMT): The functional identification of RgCOMT has elucidated its role in the biosynthesis of ferulic acid (FA), another important bioactive compound. The overexpression of RgCOMT in *R. glutinosa* significantly increased FA yield. Additionally, the reconstitution of the FA biosynthetic pathway in Saccharomyces cerevisiae using *R. glutinosa* enzymes demonstrated the catalytic abilities of RgCOMT and other related enzymes, paving the way for efficient FA production.

Dynamic Accumulation of Glycosides and Saccharides: Studies on the dynamic accumulation of glycosides and saccharides in *R. glutinosa* have revealed that the leaves, often discarded as non-medicinal parts, contain significant amounts of bioactive compounds such as catalpol, acteoside, and various carbohydrates. This finding suggests the potential for utilizing the leaves as a valuable resource.

Phytochemical Profile of Aerial Parts: The aerial parts of *R. glutinosa* have been found to contain various ursane-type triterpenoids and glycosides, including ajugol, aucubin, and acteoside. These compounds contribute to the phytochemical diversity and potential medicinal value of the aerial parts, which have been less studied compared to the roots.

The findings from these studies open several avenues for future research:

Biosynthetic Pathways: Further elucidation of the biosynthetic pathways of key bioactive compounds in *R. glutinosa* is essential. Understanding the molecular mechanisms and regulatory networks involved can lead to the development of strategies for enhancing the production of these compounds through genetic engineering or optimized cultivation practices.

Utilization of Non-Medicinal Parts: The discovery of significant bioactive compounds in the leaves of *R. glutinosa* suggests the need for comprehensive studies on the potential medicinal uses of these parts. Research should focus on the extraction, characterization, and pharmacological evaluation of compounds from the leaves to fully exploit the plant's medicinal potential.

Metabolic Engineering: The successful reconstitution of biosynthetic pathways in heterologous systems like Saccharomyces cerevisiae highlights the potential for metabolic engineering to produce bioactive compounds. Future research should explore the optimization of these systems for large-scale production and the exploration of other host organisms for biosynthesis.

Phytochemical Diversity: Continued exploration of the phytochemical diversity in different parts of *R. glutinosa*, including less-studied aerial parts, can lead to the discovery of new bioactive compounds. Advanced analytical techniques and bioinformatics tools should be employed to identify and characterize these compounds.

The research on *Rehmannia glutinosa* has significantly advanced our understanding of the biosynthesis and functional roles of its bioactive components. The identification of key enzymes such as RgTyDC2 and RgCOMT, along with the dynamic accumulation of glycosides and saccharides, underscores the plant's complex metabolic network and its potential for medicinal applications. The findings also highlight the untapped potential of the plant's non-medicinal parts, suggesting a more holistic approach to its utilization.

Future research should focus on unraveling the complete biosynthetic pathways, exploring the medicinal value of all plant parts, and leveraging metabolic engineering for the sustainable production of bioactive compounds. By doing so, we can fully unlock the therapeutic potential of *Rehmannia glutinosa* and contribute to the development of novel medicinal products.

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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.

References

Cai R.R., Zhao L.F., and Suo M.R., 2024, Active essence of Chrysanthemum morifolium: comprehensive study of chemical characteristics and bioactivity, Medicinal Plant Research, 14(1): 45-56.
https://doi.org/10.5376/mpr.2024.14.0004

Chen H., Liu X., Xie M., Zhong X., Yan C., Xian M., and Wang S., 2023, Two polysaccharides from *Rehmannia glutinosa*: isolation, structural characterization, and hypoglycemic activities, RSC Advances, 13: 30190-30201.
https://doi.org/10.1039/d3ra05677e

Dai X., Su S., Cai H., Wei D., Yan H., Zheng T., Zhu Z., Shang E., Guo S., Qian D., and Duan J., 2018, Protective effects of total glycoside from *Rehmannia glutinosa* leaves on diabetic nephropathy rats via regulating the metabolic profiling and modulating the TGF-β1 and Wnt/β-Catenin signaling pathway, Frontiers in Pharmacology, 9: 1012.
https://doi.org/10.3389/fphar.2018.01012

Dong T., Song S., Wang Y., Yang R., Chen P., Su J., Ding X., Liu Y., and Duan H., 2022, Effects of 5-azaC on iridoid glycoside accumulation and DNA methylation in *Rehmannia glutinosa*, Frontiers in Plant Science, 13: 913717.
https://doi.org/10.3389/fpls.2022.913717

Ge X., Zheng Y., He Y., Chen C., Yang C., Lu S., Xuan Z., Zhong D., and Diao X., 2023, Pharmacokinetics, mass balance, tissue distribution, and metabolism of [3H]catalpol in rats: the main bioactive component of *Rehmannia glutinosa* for the treatment of ischemic stroke, Current Drug Metabolism, 24(6): 448-457.
https://doi.org/10.2174/1389200224666230705142901

Gong W., Zhang N., Cheng G., Zhang Q., He Y., Shen Y., Zhang Q., Zhu B., Zhang Q., and Qin L., 2019, *Rehmannia glutinosa* libosch extracts prevent bone loss and architectural deterioration and enhance osteoblastic bone formation by regulating the IGF-1/PI3K/mTOR pathway in streptozotocin-induced diabetic rats, International Journal of Molecular Sciences, 20(16): 3964.
https://doi.org/10.3390/ijms20163964

Huang Y., Nan L., Xiao C., Ji Q., Li K., Wei Q., Liu Y., and Bao G., 2019, Optimum preparation method for self-assembled PEGylation nano-adjuvant based on *Rehmannia glutinosa* polysaccharide and its immunological effect on macrophages, International Journal of Nanomedicine, 14: 9361-9375.
https://doi.org/10.2147/IJN.S221398

Jeong S., Jang J., Cho H., and Lee Y., 2020, Simultaneous determination of three iridoid glycosides of *Rehmannia glutinosa* in rat biological samples using a validated hydrophilic interaction-ultrahigh performance liquid chromatography-MS/MS in pharmacokinetic and in vitro studies, Journal of Separation Science, 43(22): 4148-4161.
https://doi.org/10.1002/jssc.202000809

Kim Y., Komakech R., Jeong D., Park Y., Lee T., Kim K., Lee A., Moon B., and Kang Y., 2020, Verification of the field productivity of *Rehmannia glutinosa* (Gaertn.) DC. developed through optimized in vitro culture method, Plants, 9(3): 317.
https://doi.org/10.3390/plants9030317

Kwak M., Yu K., Lee P., and Jin J., 2018, *Rehmannia glutinosa* polysaccharide functions as a mucosal adjuvant to induce dendritic cell activation in mediastinal lymph node, International Journal of Biological Macromolecules, 120(Pt B): 1618-1623.
https://doi.org/10.1016/j.ijbiomac.2018.09.187

Kwon Y., Yu S., Choi G., Kim J., Baik M., Su S., and Kim W., 2019, Puffing of *Rehmannia glutinosa* enhances anti-oxidant capacity and down-regulates IL-6 production in RAW 264.7 cells, Food Science and Biotechnology, 28: 1235-1240.
https://doi.org/10.1007/s10068-019-00566-z

Li H., Zhang S., Zhao Y., He J., and Chen X., 2023, Identification of raffinose family oligosaccharides in processed *Rehmannia glutinosa* Libosch using matrix-assisted laser desorption/ionization mass spectrometry image combined with machine learning, Rapid Communications in Mass Spectrometry: RCM, 37(22): e9635.
https://doi.org/10.1002/rcm.9635

Li S., Fan L., Xiong D., Zhu L., Wang X., and Chen X., 2023, Compounds from *Rehmannia glutinosa* and the activity to suppress α-glucosidase, Natural Product Research, 38(12): 2060-2068.
https://doi.org/10.1080/14786419.2023.2241968

Li X., Jiang C., Xu N., Li J., Meng F., and Zhai H., 2018, Sorting and identification of *Rehmannia glutinosa* germplasm resources based on EST-SSR, scanning electron microscopy micromorphology, and quantitative taxonomy, Industrial Crops and Products, 123: 303-314.
https://doi.org/10.1016/J.INDCROP.2018.06.088

Li Y., Wang Y., Huang L., Chen C., An N., and Zheng X., 2022, Identification and functional characterization of tyrosine decarboxylase from *Rehmannia glutinosa*, Molecules, 27(5): 1634.
https://doi.org/10.3390/molecules27051634

Liu W., Yin D., Zhang T., Qiao Q., Yang Y., and Wang W., 2020, Phytochemical profiles and antioxidant activity of *Rehmannia glutinosa* from different production locations, Chemistry & Biodiversity, 17(8): e2000341.
https://doi.org/10.1002/cbdv.202000341

Liu Y., Zhang W., Zhou J., Shi G., Li X., Sun M., Chen R., and Yu D., 2023, Four new iridoid glycosides from the roots of *Rehmannia glutinosa*, Journal of Asian Natural Products Research, 26(3): 293-301.
https://doi.org/10.1080/10286020.2023.2202856

Piątczak E., Kuźma Ł., Kozłowska W., Lisiecki P., Szemraj M., Płachno B., Gonciarz W., Chmiela M., Matkowski A., and Zielińska S., 2020, Phenylethanoid and iridoid glycosides production in Rehmannia elata N.E.Brown ex Prein. in vitro shoot cultures and their biological activity, Industrial Crops and Products, 158: 113050.
https://doi.org/10.1016/j.indcrop.2020.113050

Qin Y., Wang F., Lu C., Wang F., Wen Y., Liu Y., Gao S., Qi W., Li X., and Yang J., 2022, First report of tobacco mild green mosaic virus infecting *Rehmannia glutinosa* in China, Plant Disease, 106(11): 3004.
https://doi.org/10.1094/PDIS-10-21-2283-PDN

Qin Z., Wang W., Liao D., Wu X., and Li X., 2018, UPLC-Q/TOF-MS-based serum metabolomics reveals hypoglycemic effects of *Rehmannia glutinosa*, coptis chinensis and their combination on high-fat-diet-induced diabetes in KK-Ay mice, International Journal of Molecular Sciences, 19(12): 3984.
https://doi.org/10.3390/ijms19123984

Rahmat E., Chung Y., Nam H., Lee A., Park J., and Kang Y., 2022, Evaluation of marker compounds and biological activity of in vitro regenerated and commercial *Rehmannia glutinosa* (Gaertn.) DC. roots subjected to steam processing, Evidence-based Complementary and Alternative Medicine: eCAM, 2022(1): 1506703.
https://doi.org/10.1155/2022/1506703

Tao J., Zhao M., Jiang S., Zhang W., Xu B., and Duan J., 2018, UPLC-Q-TOF/MS-based metabolic profiling comparison of four major bioactive components in normal and CKD rat plasma, urine and feces following oral administration of Cornus officinalis Sieb and *Rehmannia glutinosa* Libosch herb couple extract, Journal of Pharmaceutical and Biomedical Analysis, 161: 254-261.
https://doi.org/10.1016/j.jpba.2018.08.051

Xia Z., Li C., Hu S., Chen S., and Xu Y., 2021b, The complete chloroplast genome of Chinese medicine cultivar species of *Rehmannia glutinosa* (Orobanchaceae), Mitochondrial DNA. Part B, Resources, 6: 290-292.
https://doi.org/10.1080/23802359.2020.1863163

Xia Z., Li C., Wen J., and Wang Y., 2021a, Rehmannieae or rehmanniaceae? Evidence from plastome sequences and floral morphology, Botanical Journal of the Linnean Society, 196(2): 145-162.
https://doi.org/10.1093/BOTLINNEAN/BOAA105

Xu J., Yu Z., Li T., Song L., Qiu Z., Huang L., Chen H., and Li H., 2023, Combination of internal extractive electrospray ionization mass spectrometry and statistical analysis for high-throughput molecular differentiation of *Rehmannia glutinosa* samples, Journal of the American Society for Mass Spectrometry, 34(7): 1342-1348.
https://doi.org/10.1021/jasms.3c00043

Xu Z., Dai X., Su S., Yan H., Guo S., Qian D., and Duan J., 2019, Investigation of dynamic accumulation and regularity of nine glycosides and saccharides in *Rehmannia glutinosa* by rapid quantitative analysis technology, Journal of Separation Science, 42(8): 1489-1499.
https://doi.org/10.1002/jssc.201801185

Yang Y., Song H., Lai J., Li R., Wang Z., Jia H., and Yang Y., 2023, A *Rehmannia glutinosa* caffeic acid O-methyltransferase functional identification: Reconstitution of the ferulic acid biosynthetic pathway in Saccharomyces cerevisiae using *Rehmannia glutinosa* enzymes, Biotechnology Journal, 18(11): 2300064.
https://doi.org/10.1002/biot.202300064

Yang Y., Wang C., Li R., Zhang Z., Yang H., Chu C., and Li J., 2020, Overexpression of RgPAL family genes involved in phenolic biosynthesis promotes the replanting disease development in *Rehmannia glutinosa*, Journal of Plant Physiology, 257: 153339.
https://doi.org/10.1016/j.jplph.2020.153339

Yang Y., Yang H., Li R., Li C., Zeng L., Wang C., Li N., and Luo Z., 2021, A *Rehmannia glutinosa* cinnamate 4-hydroxylase promotes phenolic accumulation and enhances tolerance to oxidative stress, Plant Cell Reports, 40: 375-391.
https://doi.org/10.1007/s00299-020-02639-4

Zhang B., Weston P., Gu L., Zhang B., Li M., Wang F., Tu W., Wang J., Weston L., and Zhang Z., 2019, Identification of phytotoxic metabolites released from *Rehmannia glutinosa* suggest their importance in the formation of its replant problem, Plant and Soil, 441: 439-454.
https://doi.org/10.1007/s11104-019-04136-4

Zhang K., Zhuang X., Guo X., Xu H., He Z., and Chen J., 2021, Cucurbit chlorotic yellows virus infecting *Rehmannia glutinosa* was detected in China, Plant Disease, 105(10): 3310.
https://doi.org/10.1094/PDIS-02-21-0292-PDN

Zhi J., Li Y., Zhang Z., Yang C., Geng X., Zhang M., Li X., Zuo X., Li M., Huang Y., Wang F., and Xie C., 2018, Molecular regulation of catalpol and acteoside accumulation in radial striation and non-radial striation of *Rehmannia glutinosa* tuberous root, International Journal of Molecular Sciences, 19(12): 3751.
https://doi.org/10.3390/ijms19123751