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# Exploring the Genome of *Rehmannia glutinosa*: Understanding Its Genetic Code and Medicinal Potential

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**Abstract** The main objective of this study is to explore the genome of *Rehmannia glutinosa* to elucidate its genetic code and understand the underlying mechanisms responsible for its medicinal properties. By integrating genomic data with traditional knowledge, this study aims to identify key bioactive compounds and their biosynthetic pathways, as well as the genetic variability within natural populations, providing insights into breeding strategies and biotechnological applications. Genomic research on *Rehmannia glutinosa* has revealed a complex genetic landscape with significant variability among natural populations. Key bioactive compounds, including iridoids, phenylethanoids, and polysaccharides, have been identified along with their respective biosynthetic pathways. Advances in genetic engineering and tissue culture techniques have facilitated the enhancement of medicinal traits and the large-scale production of high-quality plant material. Additionally, the integration of traditional knowledge with genomic data has led to the development of more effective and standardized herbal formulations. The findings from genomic research on *Rehmannia glutinosa* provide a comprehensive understanding of its genetic code and medicinal potential. These insights pave the way for the development of improved therapeutic agents and sustainable cultivation practices. Future research should focus on overcoming current genomic limitations, exploring genetic diversity, and leveraging synthetic biology for the scalable production of bioactive compounds. The interdisciplinary approach combining traditional wisdom with modern science holds great promise for unlocking the full medicinal potential of *Rehmannia glutinosa*.
**Keywords** *Rehmannia glutinosa*; Traditional Chinese medicine; Genomic research; Bioactive compounds; Medicinal properties; Genetic diversity; Breeding strategies; Genetic engineering

## 1 Introduction

*Rehmannia glutinosa* (Gaertn.) DC, a perennial herbaceous plant belonging to the family Scrophulariaceae, holds a significant position in the realm of botanical research due to its unique genetic composition and extensive medicinal properties and has been extensively cultivated in China, Korea, Japan, and northern Vietnam (Kim et al., 2020; Zhang et al., 2021; Qin et al., 2022). It is characterized by its tubular flowers and fleshy roots, which are the primary source of its medicinal compounds. The plant is primarily propagated through vegetative means, which has led to challenges such as viral infections and reduced productivity (Kim et al., 2020; Qin et al., 2022). Recent advancements in genetic and tissue culture techniques have aimed to address these issues, enhancing the quality and yield of *R. glutinosa* (Kim et al., 2020; Li et al., 2021). The exploration of the *Rehmannia glutinosa* genome offers a profound understanding of its biological functions and evolutionary adaptations, making it a subject of intense scientific investigation.

*Rehmannia glutinosa* is native to China and has been used in traditional Chinese medicine (TCM) for over two thousand years. Its roots are utilized in various forms, including fresh rehmannia root, processed rehmannia root, and dried rehmannia root, each preparation offering distinct therapeutic effects. *Rehmannia glutinosa* is renowned for its ability to nourish yin, replenish blood, and enhance the functions of the kidneys and liver. It is frequently used in TCM prescriptions to treat a range of conditions, such as anemia, hemoptysis, diabetes, fever, and gynecological disorders (Li et al., 2018; Gong et al., 2019; Qin et al., 2022). The bioactive compounds in rehmannia, such as catalpol and acteoside, have been shown to possess pharmacological activities, including anti-diabetic and anti-osteoporotic effects (Zhi et al., 2018; Gong et al., 2019; Li et al., 2022). The medicinal

potential of rehmannia has spurred extensive research into its genetic makeup and the biosynthetic pathways of its active ingredients (Zhi et al., 2018; Chen et al., 2022; Li et al., 2022).

This study aims to delve deeply into the genetic landscape of *Rehmannia glutinos*a, providing a comprehensive analysis of its genome and elucidating the genetic basis of its medicinal properties. By integrating genomic data with traditional knowledge, this research seeks to decode the genetic secrets of *Rehmannia glutinos*, identify the key genes and pathways involved in the biosynthesis of its therapeutic compounds, and explore the evolutionary dynamics that shape its genome structure and contribute to its medicinal efficacy. Additionally, this study assesses the potential of genomic insights in the cultivation, conservation, and clinical application of *Rehmannia glutinos*. Through this systematic investigation, we hope to bridge the gap between traditional herbal wisdom and modern genomic science, fostering a deeper understanding of the genetic and medicinal potential of *Rehmannia glutinos*, and highlighting its promise in future research and therapeutic applications.

## 2 Botanical Characteristics of *Rehmannia glutinosa*

### 2.1 Morphology and taxonomy

*Rehmannia glutinosa*, a perennial herbaceous plant belonging to the family Scrophulariaceae, is widely recognized for its medicinal properties, particularly in traditional Chinese medicine (TCM) (Li et al., 2018; Zhi et al., 2018; Kim et al., 2020). The plant typically grows to a height of 30-60 cm, with basal leaves that are ovate to oblong and crenate along the margins. These leaves are covered with fine hairs, giving them a somewhat velvety texture (Kim et al., 2020). The inflorescence consists of tubular flowers that are pale yellow to purplish in color, appearing in late spring to early summer. Each flower is bilaterally symmetrical with five lobes, contributing to its characteristic appearance. *Rehmannia glutinosa* is characterized by its tuberous roots, which are the primary source of its medicinal compounds, including catalpol and acteoside (Zhi et al., 2018).

Taxonomically, *R. glutinosa* is classified within the order Lamiales. It is closely related to other genera within the Scrophulariaceae family but stands out due to its unique phytochemical profile and medicinal significance. The morphology of *R. glutinosa* includes broad leaves and a robust aerial part, with variations observed between different cultivars (Kim et al., 2020). The taxonomic classification of *R. glutinosa* has been refined through various genetic and morphological analyses, including the use of expressed sequence tags (EST) derived microsatellite markers and scanning electron microscopy (SEM) micromorphology (Li et al., 2018). These studies have led to the identification of new cultivars and a better understanding of the genetic diversity within the species (Li et al., 2018).

### 2.2 Geographic distribution and habitat

*Rehmannia glutinosa* is predominantly found in East Asia, with its native range extending across various provinces in China, including Henan, Shandong, Hebei, and Shanxi, where it has been grown for centuries due to its significant medicinal value (Li et al., 2018a; Zhang et al., 2021). The plant thrives in well-drained, fertile soils and is typically propagated through rootstock rather than seeds, which poses challenges such as root rot and reduced productivity (Kim et al., 2020). The geographic distribution of *R. glutinosa* extends beyond China to Korea, Japan, and northern Vietnam, where it is also valued for its medicinal properties (Qin et al., 2022).

The plant's habitat is characterized by temperate climates with adequate rainfall, which supports its growth and the accumulation of its bioactive compounds (Zhang et al., 2021; Qin et al., 2022). The widespread cultivation and the traditional use of *R. glutinosa* in these regions underscore its importance in herbal medicine and its potential for further agricultural and pharmacological research.

## 3 Historical and Traditional Uses

### 3.1 Traditional medicinal applications

*Rehmannia glutinosa*, a perennial herb belonging to the Scrophulariaceae family, has been a cornerstone in traditional Chinese medicine (TCM) for centuries. Its roots, known as Rehmanniae Radix (RR), are utilized in various forms, including dried and steamed preparations, to treat a multitude of ailments. Historically, RR has been employed to address conditions such as anemia, hemoptysis, and gynecological disorders (Qin et al., 2022).

The medicinal applications of *R. glutinosa* are deeply rooted in its ability to tonify the blood and nourish the yin, making it a vital component in TCM formulations (Ota et al., 2019).

### 3.2 Ethnopharmacological significance

The ethnopharmacological significance of *Rehmannia glutinosa* extends beyond its traditional uses in TCM. The plant is also recognized in Korean and Japanese traditional medicine systems, where it is valued for its tonic effects (Jeon et al., 2019). Ethnopharmacological studies have highlighted the plant's potential in modern medicine, particularly in the management of diabetes-induced osteoporosis.

Gong et al. (2019) used *R. glutinosa* extract on streptozotocin (STZ)-induced diabetic rats to observe its effects on bone density, bone microstructure, and biochemical markers. The results showed that Rehmannia extract significantly increased bone density and improved bone microstructure in diabetic rats, enhanced the activity of bone formation marker ALP, and reduced the levels of bone resorption marker OCN. CAT, ACT, and ECH significantly promoted the proliferation and differentiation of osteoblasts damaged by high glucose and enhanced bone formation by regulating the IGF-1/PI3K/mTOR signaling pathway (Figure 1). Extracts from *R. glutinos*a have been shown to prevent bone loss and enhance osteoblastic bone formation by regulating the IGF-1/PI3K/mTOR pathway, demonstrating its relevance in contemporary therapeutic contexts (Gong et al., 2019). Additionally, the processing methods of RR, such as steaming and pretreatment with liquor, have been investigated for their impact on the plant's immunostimulatory effects, further underscoring its pharmacological importance (Ota et al., 2019).

## 4 Genomic Studies of *Rehmannia glutinosa*

### 4.1 Overview of genomic research

*Rehmannia glutinosa*, a perennial herb widely used in traditional Chinese medicine, has been the subject of various genomic studies aimed at understanding its genetic makeup and medicinal properties (Kim et al., 2020). These studies have employed advanced genomic techniques to explore the plant's gene content, structure, and functional pathways, providing valuable insights into its biological and pharmacological characteristics.

### 4.2 Methodologies in genome sequencing

The extraction of high-quality DNA is a critical step in genome sequencing. In the case of *Rehmannia glutinosa*, various sequencing technologies have been employed (Gong et al., 2019) For instance, the use of next-generation sequencing (NGS) has been pivotal in confirming gene editing events, such as the CRISPR/Cas9-mediated knockout of the phytoene desaturase (PDS) gene, which resulted in albino plants (Li et al., 2021). Additionally, whole-genome Illumina sequencing has been utilized to complete the chloroplast genomes of *R. glutinos*a, revealing significant intra-species diversity (Jeon et al., 2019).

Genome assembly and annotation involve piecing together the sequenced fragments and identifying functional elements within the genome. In Rehmannia glutinosa, de novo assembly techniques have been used to construct comprehensive transcriptomes, which have facilitated the identification of key genes involved in the biosynthesis of medicinal compounds such as catalpol and acteoside (Zhi et al., 2018). Furthermore, the annotation of chloroplast genomes has provided insights into the genetic diversity and phylogenetic relationships within the species (Jeon et al., 2019).

### 4.3 Key Findings from genome sequencing

Genomic studies have revealed a wealth of information about the gene content and structure of *Rehmannia glutinosa*. For example, the identification and characterization of PAL family genes have shown their involvement in phenolic biosynthesis, which is crucial for the plant's medicinal properties (Yang et al., 2020). Additionally, the sequencing of chloroplast genomes has identified 114 coding regions, including 80 protein-coding genes, 4 rRNA genes, and 30 tRNA genes, highlighting the complexity of the plant's genetic makeup (Jeon et al., 2019).

Comparative genomics has been instrumental in understanding the evolutionary relationships and genetic diversity within the *Rehmannia* genus. Studies have shown that *R. glutinosa* shares a close phylogenetic relationship with

other *Rehmannia* species, as evidenced by the clustering of chloroplast genomes (Jeon et al., 2019). Moreover, DNA barcoding techniques have been employed to distinguish between different *Rehmannia* species and cultivars, providing a robust framework for species identification and classification (Duan et al., 2019).

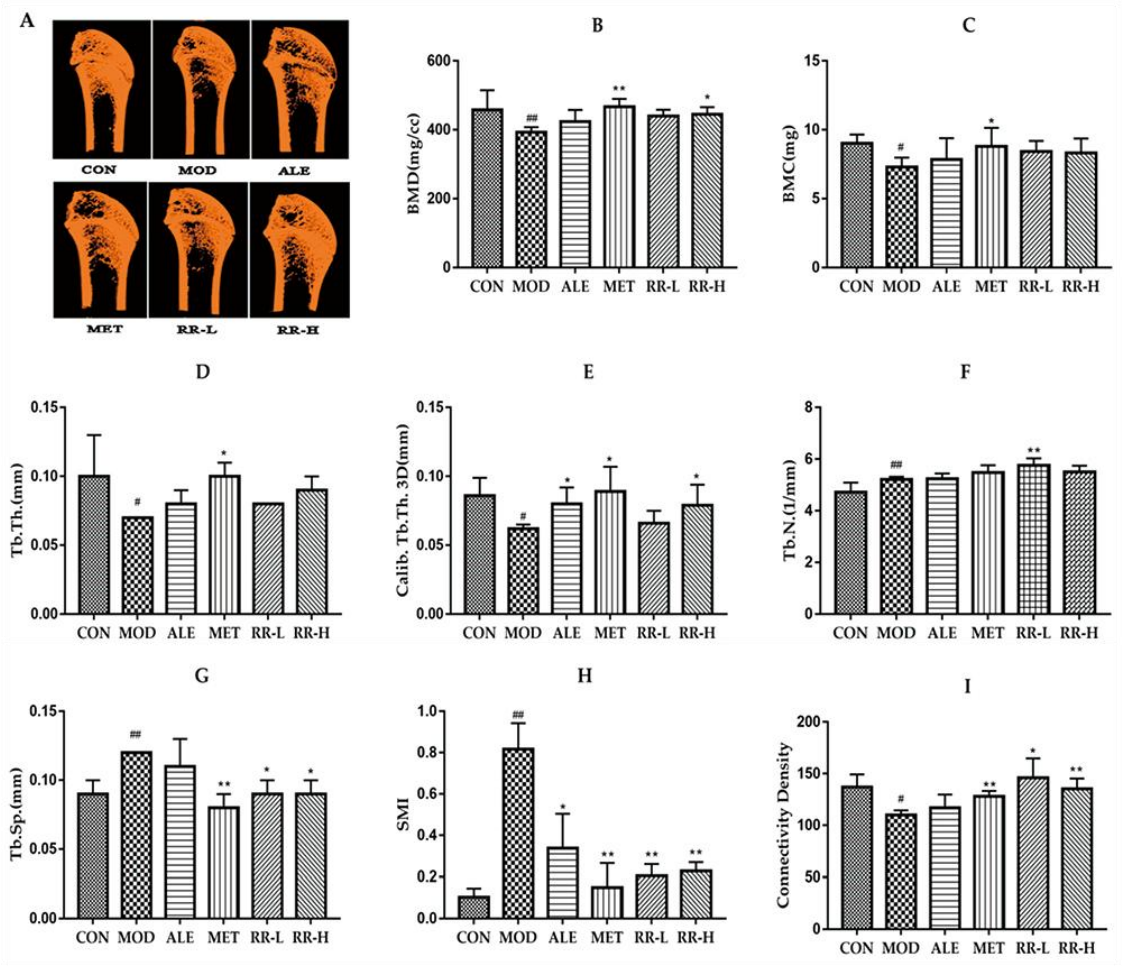


Figure 1 Effects of RR on bone mineral density and histological morphometric alteration of the femurs in diabetic rats (Adopted from Gong et al., 2019)

Image caption: A: Representative 3D micro-CT images of the distal femoral trabecular bone in diabetic rats. The results show that compared to the normal control group, the bone mass in diabetic rats is significantly reduced and the bone microarchitecture is deteriorated. After 8 weeks of treatment with *Rehmannia glutinosa* extract (RR), the bone structure deterioration was effectively prevented; B: Changes in bone mineral density (BMD), indicating that RR treatment significantly increased the BMD of diabetic rats, with a significant difference compared to the model group ($p < 0.01$). C: Changes in bone mineral content (BMC). The BMC in the RR treatment group was significantly higher than that in the model group ($p < 0.01$), indicating that RR could increase bone mineral content; D: Changes in trabecular thickness (Tb.Th). RR treatment significantly increased trabecular thickness ($p < 0.01$), showing a significant difference compared to the model group; E: Changes in 3D-calibrated trabecular thickness (calib.Tb.Th.3D). After RR treatment, trabecular thickness was significantly increased ($p < 0.01$), indicating the positive regulatory effect of RR on trabecular thickness; F: Changes in trabecular number (Tb.N). The trabecular number in the RR treatment group was significantly higher than that in the model group ($p < 0.01$), indicating that RR could increase the number of trabeculae; G: Changes in trabecular separation (Tb.Sp). RR treatment significantly reduced trabecular separation ($p < 0.01$), indicating that RR helps to decrease the distance between trabeculae; H: Changes in the structure model index (SMI). After RR treatment, SMI was significantly reduced ($p < 0.01$), indicating a more stable bone structure; I: Changes in connectivity density. The connectivity density in the RR treatment group was significantly higher than that in the model group ($p < 0.01$), indicating that RR could improve the connectivity of the bone structure. The results in the figure demonstrate that *Rehmannia glutinosa* extract can significantly improve the bone density and bone microarchitecture in diabetic rats, validating its potential mechanism in preventing and treating diabetic osteoporosis through the regulation of the IGF-1/PI3K/mTOR signaling pathway(Adapted from Gong et al., 2019)

## 5 Bioactive Compounds and Their Biosynthesis

### 5.1 Identification of key bioactive compounds

Iridoids are a significant class of bioactive compounds found in *Rehmannia glutinos*a. These compounds are known for their diverse pharmacological activities, including anti-inflammatory, antioxidant, and neuroprotective effects. Several studies have identified key iridoid glycosides in *R. glutinosa*, such as catalpol and ajugol, which are present in both the roots and leaves of the plant (Xu et al., 2019; Dong et al., 2022). The biosynthesis of iridoids involves the enzyme iridoid synthase (IS), which catalyzes the cyclization of 10-oxogeranial to epi-iridodial, a crucial step in the iridoid biosynthetic pathway (Yang et al., 2019).

Phenylethanoids, including acteoside and other phenylethanoid glycosides, are another important group of bioactive compounds in *R. glutinosa*. These compounds exhibit various biological activities, such as antioxidant, anti-inflammatory, and hepatoprotective effects. The phenylpropanoid pathway, which involves enzymes like phenylalanine ammonia-lyase (PAL) and cinnamate 4-hydroxylase (C4H), is critical for the biosynthesis of phenylethanoids (Yang et al., 2020; 2021; 2023).

Polysaccharides in *R. glutinosa* are known for their immunomodulatory and antitumor activities. These complex carbohydrates, including acidic and neutral polysaccharides, are found in significant amounts in both the roots and leaves of the plant. The dynamic accumulation of these polysaccharides varies with the growth stages and cultivation regions of *R. glutinosa* (Xu et al., 2019).

### 5.2 Biosynthetic Pathways of Major Compounds

The biosynthesis of iridoids, phenylethanoids, and polysaccharides in *R. glutinosa* involves several key enzymes and genes. For iridoids, enzymes such as 1-deoxy-D-xylulose 5-phosphate synthase (DXS), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), and geranyl diphosphate synthase (GPPS) play crucial roles (Yang et al., 2019; Dong et al., 2022). In the phenylpropanoid pathway, enzymes like PAL, C4H, and p-coumarate 3-hydroxylase (C3H) are essential for the biosynthesis of phenylethanoids (Yang et al., 2020; 2021; 2023). The biosynthesis of polysaccharides involves various glycosyltransferases and other enzymes responsible for the polymerization of monosaccharides (Xu et al., 2019).

The regulation of biosynthetic pathways in *R. glutinosa* is complex, involving multiple levels such as gene expression, enzyme activity, and epigenetic modifications. For example, Dong et al. (2022) explored the effects of 5-azacytidine (5-azaC) on the accumulation of iridoid glycosides and DNA methylation in *R. glutinosa*. The study showed that 5-azaC treatment significantly increased the expression levels of genes related to iridoid glycoside synthesis in *Rehmannia glutinosa*, including DXS, DXR, GPPS, G10H, and 10HGO. This upregulation of gene expression corresponded with the accumulation of iridoid glycosides, particularly under the treatment with 50 μM 5-azaC (Figure 2). Additionally, 5-azaC induced DNA demethylation in *Rehmannia glutinosa*, displaying a dose-dependent response.

Similarly, the overexpression of PAL and C4H genes has been shown to enhance the production of phenylethanoids and improve the plant's tolerance to oxidative stress (Yang et al., 2020; 2021; 2023). The differential expression of homologous genes encoding key enzymes also plays a significant role in the regulation of terpenoid and phenylethanoid biosynthesis (Kang et al., 2022).

## 6 Pharmacological Activities and Mechanisms

### 6.1 Antioxidant properties

*Rehmannia glutinosa* exhibits significant antioxidant properties, which are primarily attributed to its phenolic compounds. The cinnamate 4-hydroxylase (C4H) gene in *R. glutinosa* promotes phenolic accumulation, enhancing the plant's tolerance to oxidative stress by activating its antioxidant systems (Yang et al., 2021). Additionally, the antioxidant activity of *R. glutinosa* has been demonstrated through various assays, showing high levels of catalpol, rehmaionoside A, and rehmannioside D, which contribute to its strong antioxidant capacity (Liu et al., 2020). The antioxidant effects are further supported by the increased activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in treated models (Li et al., 2023).

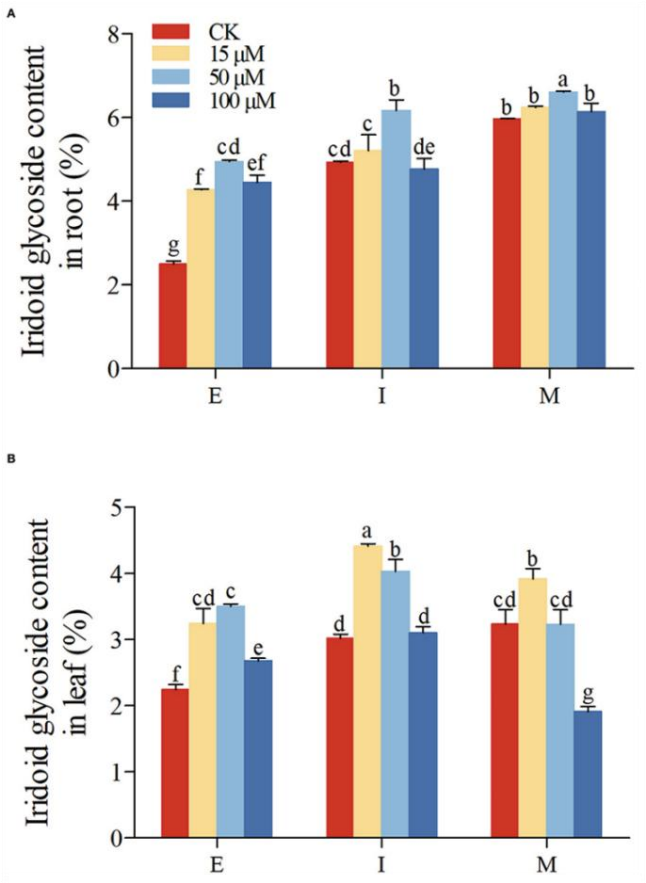


Figure 2 Effect of 5-azaC on iridoid glycoside accumulation in *R. glutinosa* (Adopted from Dong et al., 2022)

Image caption: (A) and (B) respectively show the content of iridoid glycosides in the roots and leaves of *R. glutinosa* at different growth stages (E, I, M). The results indicate that 5-azaC treatment significantly increased the content of iridoid glycosides in the roots at all growth stages, with the most pronounced effect observed at the 50 μM treatment. In the leaves, the accumulation of iridoid glycosides also increased, although not as significantly as in the roots. This suggests that 5-azaC plays an important role in regulating the accumulation of secondary metabolites in *R. glutinosa* (Adapted from Dong et al., 2022)

### 6.2 Anti-inflammatory effects

*Rehmannia glutinosa* also possesses significant anti-inflammatory properties. The oligosaccharides (RGO) isolated from *Rehmannia glutinosa* have been shown to combat lipopolysaccharide (LPS)-induced intestinal inflammation by reducing the levels of inflammatory cytokines. The study by Li et al. (2023) indicates that the oligosaccharides extracted from *Rehmannia glutinosa* (RGO) provide significant protection against LPS-induced intestinal inflammation and barrier damage in a mouse model. RGO decreased the levels of inflammatory cytokines (e.g., IL-6, IL-17, IL-1β, and TNF-α) in intestinal tissues, increased the activities of antioxidant enzymes (SOD, GSH-Px, and CAT), and reduced the content of MDA (Figure 3). Furthermore, the polysaccharides from *R. glutinosa* attenuate colitis by inhibiting the NF-κB pathway, thereby reducing the expression of inflammatory factors and improving intestinal health (Lv et al., 2022). The anti-inflammatory activity is also evident in the suppression of nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression in various models (Rahmat et al., 2022).

### 6.3 Neuroprotective actions

The neuroprotective effects of *Rehmannia glutinos*a are largely attributed to catalpol, an iridoid glycoside found in the plant. Catalpol has been shown to protect against spinal cord injury by inhibiting endoplasmic reticulum (ER) stress-mediated neuronal apoptosis, thereby preserving motor function and reducing neuronal death (Huang et al., 2022). Additionally, catalpol's neuroprotective mechanisms include the elevation of serotonin and brain-derived neurotrophic factor (BDNF) levels, which protect against depression and neurodegeneration (Bhattamisra et al., 2019).

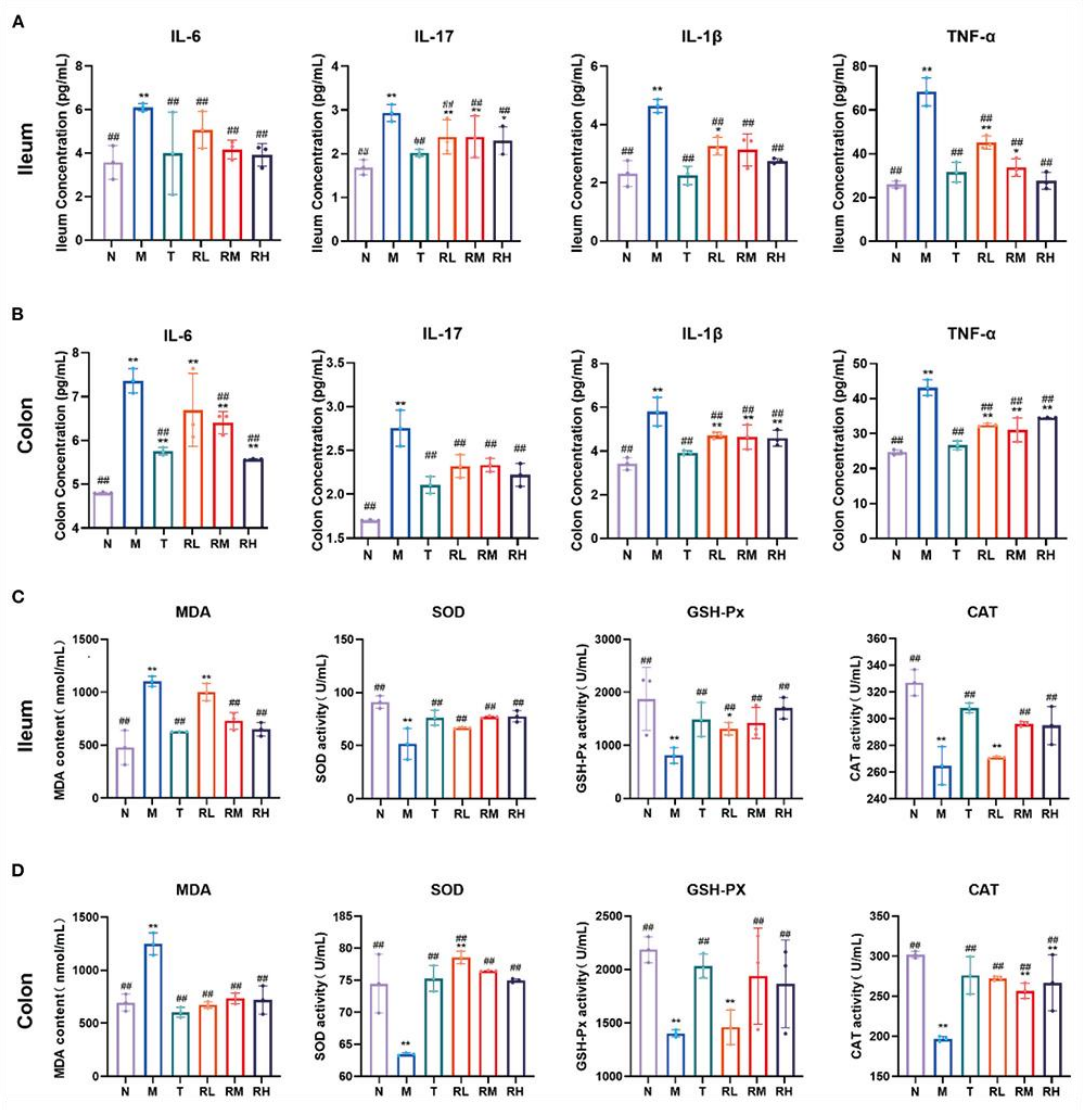


Figure 3 The effect of RGO on intestinal inflammatory factors and oxidative indexes in LPS mice (Adopted from Li et al., 2023)

Image caption: A and B show that LPS treatment significantly increased the levels of IL-6, IL-17, IL-1β, and TNF-α in the intestinal tissues of mice compared to the normal group, while the RGO treatment group significantly reduced these inflammatory cytokines. Figures C and D indicate that LPS treatment significantly elevated the levels of MDA in the intestinal tissues of mice and decreased the activities of SOD, GSH-Px, and CAT. In contrast, the RGO treatment group significantly restored the activities of these antioxidant enzymes and reduced the levels of MDA. These results suggest that RGO can alleviate LPS-induced intestinal inflammation and oxidative damage by reducing inflammatory cytokines and enhancing antioxidant capacity (Adapted from Li et al., 2023)

### 6.4 Other pharmacological activities

*Rehmannia glutinosa* exhibits a range of other pharmacological activities, including anti-diabetic, cardiovascular protective, and hepatoprotective effects. The anti-diabetic properties are linked to the activation of the PI3K/Akt pathway, which enhances insulin sensitivity (Bhattamisra et al., 2019). The cardiovascular protective effects involve the apelin/APJ and Jak-Stat pathways, which contribute to improved heart health (Bhattamisra et al., 2019). Additionally, the plant's hepatoprotective effects are demonstrated through its ability to reduce liver damage and improve liver function in various models (Bhattamisra et al., 2019).

*Rehmannia glutinosa* demonstrates a wide array of pharmacological activities, including antioxidant, anti-inflammatory, neuroprotective, anti-diabetic, cardiovascular protective, and hepatoprotective effects. These activities are mediated through various molecular mechanisms, highlighting the plant's potential as a valuable medicinal resource.

# 7 Genetic Diversity and Breeding

## 7.1 Genetic variability in natural populations

Rehmannia glutinosa, a valuable medicinal plant, exhibits significant genetic diversity across its natural populations. Studies have utilized various molecular markers to assess this diversity. For instance, the use of SRAP molecular markers has revealed a medium-low level of genetic diversity among 21 species of R. glutinosa, with polymorphic loci percentages ranging from 8.77% to 54.39% and a Nei's genetic diversity index (H) of 0.374 1 (Shi et al., 2018). Additionally, expressed sequence tags (EST) derived microsatellite markers have been employed to confirm genetic relationships among 25 core germplasms, leading to the identification of four new cultivars (Li et al., 2018). These findings underscore the importance of genetic variability in the conservation and breeding of *R. glutinosa*.

## 7.2 Breeding strategies for enhanced medicinal traits

### 7.2.1 Traditional breeding techniques

Traditional breeding techniques in *R. glutinosa* have primarily focused on the selection and cultivation of superior germplasms. Quantitative taxonomy and micromorphology analysis have been used to identify and classify different germplasms, aiding in the selection of high-yield and high-quality cultivars (Li et al., 2018). Additionally, the evaluation of genetic diversity and medicinal quality through molecular markers and HPLC analysis has provided theoretical guidance for screening excellent germplasms, such as those with higher contents of catalpol and verbascoside (Shi et al., 2018). These traditional methods have been instrumental in the development of new cultivars with enhanced medicinal traits.

### 7.2.2 Modern biotechnological approaches

Modern biotechnological approaches have significantly advanced the breeding of *R. glutinos*a. The application of CRISPR/Cas9-mediated genome editing has enabled precise modifications in the *R. glutinosa* genome, such as the knockout of the phytoene desaturase (PDS) gene, resulting in the generation of albino plants and demonstrating high editing efficiency (Li et al., 2021). Furthermore, transcriptome sequencing has facilitated the identification of key genes and transcriptional regulators involved in the biosynthesis of important medicinal compounds like catalpol and acteoside, providing valuable insights for genetic improvement (Zhi et al., 2018). These biotechnological advancements hold great promise for the development of *R. glutinos*a cultivars with superior medicinal properties.

In conclusion, the integration of traditional breeding techniques with modern biotechnological approaches offers a comprehensive strategy for enhancing the genetic diversity and medicinal potential of *Rehmannia glutinosa*. By leveraging the strengths of both methods, researchers can develop new cultivars that meet the growing demand for high-quality medicinal plants.

# 8 Biotechnological Applications

## 8.1 Genetic engineering for improved compound production

Genetic engineering has been a pivotal tool in enhancing the production of valuable compounds in *Rehmannia glutinosa*. One notable example is the overexpression of the RgPAL family genes, which are involved in phenolic biosynthesis. This genetic modification has been shown to increase the production of phenolic compounds, although it also exacerbates replanting disease due to the release of allelopathic phenolics (Yang et al., 2020a). Similarly, the *RgC3H* gene, another key player in the phenolic acid/phenylpropanoid biosynthesis pathway, has been identified and manipulated to alter the release of allelopathic phenolic acids, further elucidating the molecular mechanisms underlying replanting disease (Yang et al., 2020b). Additionally, the CRISPR/Cas9 system has been successfully applied to *R. glutinosa*, demonstrating high editing efficiency and paving the way for future genetic modifications aimed at improving yield and quality (Li et al., 2021).

## 8.2 Tissue culture and *in vitro* propagation

Tissue culture and in vitro propagation techniques have been optimized to address the challenges associated with traditional cultivation methods of *R. glutinosa*. These methods have been particularly effective in producing sterile

culture seedlings and rootstocks, which are crucial for mass production. Kim et al. (2020) found that there are significant differences in the field growth characteristics and yield of medicinal parts among standard rootstock seedlings (SR), culture rootstock seedlings (CR), and culture seedlings (CS). SR had the highest number of leaves, but the leaf area and length were smaller compared to CR and CS. Additionally, the fresh and dry weights of the underground parts of CR and CS were twice that of SR. Chemical analysis showed that the chemical composition of CR and CS was similar to that of SR, but the catalpol content was slightly lower. The research indicates that CR and CS seedling types have significant advantages in large-scale field production, helping to reduce labor and increase production efficiency (Figure 4) (Kim et al., 2020). This approach not only enhances productivity but also mitigates issues such as root rot and low-quality yields, which are common in traditional seed cultivation.


Figure 4 Comparison of root morphology of the different seedling types (Adopted from Kim et al., 2020)

Image caption: Comparison of root morphology of three types of Rehmannia glutinosa seedlings (Standard Rootstock SR, Culture Rootstock CR, and Culture Seedling CS). The results showed that SR had the longest tuberous root length, while CS had the shortest root length. CR had the highest fresh weight of the tuberous root, followed by CS, and SR had the lowest. The dry weight trend was consistent with the fresh weight. CR had the largest root surface area (46 cm²), while SR had the smallest (30.3 cm²). Figure demonstrates that CR has significant advantages in field production by showing the root growth characteristics of different seedling types(Adapetd Kim et al., 2020)

### 8.3 Metabolic engineering and synthetic biology

Metabolic engineering and synthetic biology offer promising avenues for enhancing the medicinal potential of *R. glutinosa*. For instance, the biosynthetic pathways of key compounds like catalpol and acteoside have been partially elucidated through transcriptome sequencing, identifying numerous genes and transcription factors involved in their biosynthesis (Zhi et al., 2018). Furthermore, the reconstitution of the ferulic acid (FA) biosynthetic pathway in *Saccharomyces cerevisiae* using *R. glutinosa* enzymes has demonstrated the potential for producing pharmacologically significant compounds in a heterologous host (Yang et al., 2023). This approach not only facilitates the study of complex biosynthetic pathways but also enables the scalable production of valuable medicinal compounds.

The integration of genetic engineering, tissue culture, and metabolic engineering techniques holds significant promise for advancing the cultivation and medicinal application of *Rehmannia glutinosa*. These biotechnological applications not only enhance the understanding of its genetic code but also unlock new potentials for its use in traditional and modern medicine.

## 9 Challenges and Future Directions

### 9.1 Current limitations in genomic research

Despite the significant advancements in genomic research on *Rehmannia glutinosa*, several challenges remain (Zhi et al., 2018). One of the primary limitations is the incomplete and fragmented nature of the current genome assemblies. High-quality, fully assembled genomes are essential for accurately identifying and characterizing

genes and regulatory elements involved in the biosynthesis of bioactive compounds. Additionally, the complexity of the Rehmannia glutinosa genome, including the presence of repetitive sequences and polyploidy, poses difficulties in sequencing and assembly processes (Jeon et al., 2019).

Another challenge is the limited functional annotation of the genome. While many genes can be predicted based on sequence homology, the specific functions of a large number of genes remain unknown (Duan et al., 2019). This gap hinders the understanding of the biosynthetic pathways and regulatory networks that govern the production of medicinal compounds. The genetic diversity within natural populations of *Rehmannia glutinosa* is not fully explored as well, limiting the potential to harness this variability for breeding and biotechnological applications.

### 9.2 Prospects for genomic-driven drug discovery

Genomic research holds great promise for the discovery and development of new drugs from *Rehmannia glutinosa*. By leveraging advanced genomic tools, researchers can identify novel bioactive compounds and elucidate their biosynthetic pathways. High-throughput sequencing and bioinformatics analyses enable the identification of candidate genes involved in the production of therapeutic compounds. These genes can be targeted for overexpression or modification to enhance the yield and potency of the desired compounds.

Moreover, integrating genomic data with metabolomics and transcriptomics can provide a comprehensive understanding of the metabolic networks in *Rehmannia glutinosa*. This systems biology approach can uncover the regulatory mechanisms controlling the synthesis of bioactive molecules, facilitating the development of more effective and targeted therapeutic agents (Lempp et al., 2019). Genomic-driven drug discovery also opens up the possibility of producing complex plant-derived compounds in microbial systems through synthetic biology, offering a sustainable and scalable alternative to traditional plant extraction methods.

### 9.3 Integrating genomic data with traditional knowledge

Integrating genomic data with traditional knowledge is a critical step toward maximizing the medicinal potential of *Rehmannia glutinosa*. Traditional Chinese Medicine (TCM) has a long history of using *Rehmannia glutinosa* in various formulations, and this ethnobotanical knowledge provides valuable insights into the therapeutic applications of the plant. By correlating genomic data with the traditional uses and preparation methods, researchers can validate and optimize the medicinal properties of *R. glutinosa* (Huang et al., 2018; Jeon et al., 2019). Collaborative efforts between genomic scientists and practitioners of TCM can lead to the development of standardized, high-efficacy herbal products. Genomic data can inform the selection of plant varieties with optimal bioactive profiles, while traditional knowledge can guide the appropriate processing and formulation techniques. This integrative approach ensures that the benefits of modern genomics are harnessed without losing the rich cultural heritage and empirical wisdom embedded in traditional medicine (Jeon et al., 2019). Furthermore, the integration of genomic data with traditional knowledge can support conservation efforts. Understanding the genetic diversity and population structure of *Rehmannia glutinosa* can aid in the development of strategies to preserve its genetic resources and ensure sustainable use. By protecting the genetic diversity of this valuable medicinal plant can safeguard its potential for future generations.

While there are challenges in the genomic research of *Rehmannia glutinosa*, the prospects for genomic-driven drug discovery and the integration of genomic data with traditional knowledge are promising. Addressing the current limitations and fostering interdisciplinary collaborations will pave the way for realizing the full medicinal potential of *Rehmannia glutinosa*, benefiting both modern and traditional medicine.

## 10 Concluding Remarks

The exploration of the genome of *Rehmannia glutinosa* provided significant insights into its genetic code and medicinal potential. Key findings include the identification and characterization of PAL family genes involved in phenolic biosynthesis, which play a crucial role in the development of replanting disease due to autotoxic harm. The successful application of CRISPR/Cas9-mediated genome editing in *R. glutinosa* has demonstrated the potential for genetic improvements and the creation of superior germplasm. Additionally, the study of germplasm

resources using EST-SSR markers and quantitative taxonomy has led to the identification of new cultivars, enhancing the genetic diversity and breeding potential of *R. glutinosa*. The complete sequencing of chloroplast genomes has revealed significant intra-species diversity, contributing to our understanding of the genetic relationships within the species. Proteomic analysis has highlighted the complex regulation of terpenoid synthesis, a key medicinal component of *R. glutinosa*. Furthermore, shifts in the rhizosphere microbial community under consecutive monoculture have been linked to changes in soil health and plant performance, impacting the quality and yield of *R. glutinosa*.

The genetic and molecular insights gained from these studies have profound implications for the medicinal use of *Rehmannia glutinosa*. The identification of key genes involved in phenolic and terpenoid biosynthesis can lead to the development of genetically enhanced varieties with higher concentrations of these bioactive compounds, potentially increasing their therapeutic efficacy. The ability to manipulate the genome using CRISPR/Cas9 technology opens up new avenues for improving the yield and quality of *R. glutinosa*, making it more viable for large-scale medicinal production. Understanding the genetic diversity and relationships among different germplasms can aid in the selection and breeding of superior cultivars, ensuring a consistent supply of high-quality medicinal material. Additionally, insights into the rhizosphere microbiome and its impact on plant health can inform better agricultural practices, reducing the incidence of replanting disease and improving overall crop productivity.

Future research should further elucidate the molecular mechanisms of key medicinal compound biosynthesis in *R. glutinosa*, advancing the development of advanced genome editing technologies and their application in *R. glutinosa* to create new varieties with enhanced medicinal properties and disease resistance. Expanding research on the genetic and phenotypic traits of *R. glutinosa* germplasm will improve yield and quality. A more comprehensive study of rhizosphere microbial communities and their interactions with *R. glutinosa* will be crucial for developing sustainable cultivation practices, mitigating the negative impacts of continuous monoculture. Integrating these genetic and microbiological insights with traditional knowledge of *R. glutinosa*'s medicinal uses will aid in developing more effective and reliable herbal medicines.

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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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