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CRISPR Applications in Enhancing Medicinal Plant Yields

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Abstract

Original Research Article

***Background*:** Medicinal plants play a vital role in traditional and modern pharmacology due to their bioactive compounds. However, natural limitations such as low yield, long growth cycles, and vulnerability to environmental stresses hinder their large-scale cultivation. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has emerged as a transformative tool in genome editing, offering precision, efficiency, and flexibility for enhancing crop ***Objective*:** The current research seeks to examine how CRISPR gene-editing technology may be used to enhance the quality and yield of medicinal plants through the targeting of characteristics like secondary metabolite yield, disease resistance, and drought tolerance. ***Methodology*:** A review-based approach was used, examining peer-reviewed publications from the period 2018-2024. Sources were obtained from PubMed, Scopus, and Web of Science, targeting experimental and applied research that made use of CRISPR to edit the genomes of medicinal plants. Important genes associated with growth, resistance, and biosynthesis of pharmacologically active compounds were identified and explored. ***Results*:** Data indicate that CRISPR has been successfully used to increase yield and secondary metabolite content in crops such as Artemisia annua, Catharanthus roseus, and Withania somnifera. Gene insertions and targeted knockouts enhanced stress resistance and lowered pathogen susceptibility without affecting the viability of plants. ***Conclusion*:** CRISPR provides a strong pathway to improve medicinal plant cultivation for drug application. Its accurate genome-editing capability can result in more environment-friendly cultivation and conservation of precious species. Regulatory transparency and additional research will be required to incorporate CRISPR-edited medicinal plants into common healthcare and agriculture practices.

Keywords: CRISPR-Cas technology, Medicinal plants, Genome editing, Secondary metabolites, Drought tolerance.

INTRODUCTION

Medicinal plants have been the bedrock of traditional medicine systems for centuries and remain important sources of bioactive compounds for drug development (Efferth & Greten, 2021). However, yields of native plants are often low due to genetic, environmental and physiological constraints. This presents major challenges when trying to meet the increasing demand for plant therapeutic agents worldwide.

Current biotechnological tools offer ways to enhance productivity and efficiency of medicinal plants. Among these tools, CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated proteins) technology is a groundbreaking genome editing system that enables highly efficient and targeted modifications in genomes with precision at defined genomic locations (Kumari *et al.*, 2022). Originally described as an immune system found in bacteria, CRISPR-Cas9 has changed the face of genetic engineering in several species of plants from model and non-model species.

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CRISPR has been useful in crops to increase yield, resist stress, and improve nutrition (Chen *et al*., 2019). Recently CRISPR has been used in medicinal plants, with great success in enhancing biosynthesis of pharmacologically active metabolites of significance. For instance, a notable example in medicinal plants is modification of the ADS gene in Artemisia annua to enhance content of artemisinin (Li *et al*., 2020), and CRISPR-cas9-mediated control of WRKY transcription factors in Withania somnifera enhanced withanolide biosynthesis (Sharma *et al*., 2021).

Furthermore, CRISPR can edit biosynthetic pathways and regulatory genes for secondary metabolite production, which is usually of particular interest in medicinal plants (Zhou *et al*., 2021). This method of precise editing improves metabolite yield, and minimizes the need for overharvesting natural populations; thus supporting species conservation (Alok *et al*., 2020).

Although promising, there remain issues regarding the optimization of delivery systems, off-target effects, and regulatory difficulties especially among the non-model medicinal plants that have recalcitrant genomes (Naim *et al*., 2022). Nevertheless, the use of CRISPR in medicinal plant biotechnology provides a sustainable avenue to the improvement of natural product harvests, upholding pharmaceutical innovation, as well as meeting global health demands.

LITERATURE REVIEW

CRISPR/Cas systems developed so quickly from a rudimentary gene-editing tool to a versatile platform for plant metabolic engineering, particularly in plants that are medicinally important. CRISPR has enabled scientists to avoid the capabilities of traditional breeding and mutagenesis and make targeted changes in growth, resistance, and secondary metabolite production genes (Karkute *et al*., 2022).

In Salvia miltiorrhiza, a well-known medicinal herb in traditional Chinese medicine, CRISPR/Cas9 was used to knockout the SmCYP76AH1 gene, which improved the yield of tanshinones—molecules with cardiovascular advantages (Zhou *et al*., 2020). The research proved that specific knockdown of downstream negative regulators of metabolic pathways can dramatically enhance compound yield without affecting plant viability.

Similarly, Papaver somniferum (the opium poppy) is another plant which produces morphine and other alkaloids that can be efficiently edited through CRISPR to assist in deregulating genes important to the production of synthetic precursors like STORR and CYP82Y1 (Li *et al*. 2021). This type of editing demonstrates CRISPR's ability to manipulate complex pathways for improved yields of medicinal compounds.

Recent developments have also been positive for Camptotheca acuminata (of the family Nyssaceae), a plant containing the anticancer alkaloid camptothecin, which is cultivated in southern China for this compound. Editing Ca7g14120 and CaTDC1 genes using CRISPR to promote camptothecin biosynthesis were developed and tested as a new method for enhancing the commercial value of this plant (Xie *et al*., 2022).

Research on Glycyrrhiza uralensis (licorice) has also demonstrated how CRISPR can control glycyrrhizin synthesis by editing UDP-glucuronosyltransferase (UGT) genes (Yuan *et al*., 2023). All the examples of case studies demonstrate the expanding utilization of the CRISPR system in medicinal plant biotechnology.

The literature also mentions the impediments to CRISPR adoption. Many medicinal plants with complex or poorly annotated genomes pose difficulties with gene target identification (Ahmad *et al*., 2021). In addition, transformation protocol for many medicinal plants still remains inefficient or genotype-dependent (Tang *et al*., 2023).

Nevertheless, CRISPR is pushing the beginnings of its frontier. Newer technologies CRISPR/Cas12 and base-editors will provide more precision with less off-target effects, opening up new methods for generating and improvement traits for high value plant traits (Wang *et al*., 2022).

METHODOLOGY

In this research, systematic review was utilized to provide a comprehensive overview of the prospective applications of CRISPR-based genome editing technologies to increase the agronomical yield and pharmacological value of medicinal plants. The systematic review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) to assure rigor and transparency of methodology.

Data Sources and Search Strategy

Relevant literature was identified by searching three major scientific databases: PubMed, Web of Science, and Scopus. The search was conducted between January 2024 and May 2025 using a combination of keywords and Boolean operators: ("CRISPR" OR "CRISPR/Cas9" OR "genome editing") AND ("medicinal plants" OR "phytochemicals" OR "secondary metabolites") AND ("yield improvement" OR "biosynthesis" OR "metabolic engineering").

Only peer-reviewed journal articles published between 2018 and 2025 were included to ensure the currency and relevance of the data.

Inclusion and Exclusion Criteria

Studies were included in the review if they:

- Focused specifically on medicinal plants with known therapeutic compounds.
- Reported original experimental results involving CRISPR-based genome editing.
- Targeted genes related to yield, metabolite biosynthesis, or stress resistance.
- Were published in English-language journals.

Studies were excluded if they:

- Focused solely on food crops or non-medicinal species.
- Lacked CRISPR-based experimental evidence.
- Were reviews, commentaries, or editorials.

Data Extraction and Analysis

A data extraction form was developed to collect information from each selected study systematically. The following variables were extracted:

- Plant species name
- Target gene(s) edited
- CRISPR system used (e.g., Cas9, Cas12a)
- Editing method and delivery system
- Observed outcomes in terms of yield or metabolite concentration

Each study was evaluated for methodological quality, reproducibility, and clarity of results. Studies were categorized based on the primary goal of gene editing: (1) enhancement of bioactive compound yield, (2) stress tolerance improvement, or (3) resistance to pathogens.

Quality Assessment

The Joanna Briggs Institute (JBI) Critical Appraisal Checklist for experimental studies was used to assess the quality of the included studies. Articles that did not meet at least five out of seven quality criteria were excluded from the final synthesis.

Synthesis Approach

A narrative synthesis was used to summarize and interpret the findings due to the heterogeneity of plant species, gene targets, and editing outcomes. The review aimed to identify trends, gaps, and future directions for CRISPR applications in medicinal plant biotechnology.

RESULTS

Overview of Study Selection

Out of 472 initially identified records, 396 articles remained after removing duplicates. After screening titles and abstracts, 98 full-text articles were reviewed. A total of 51 studies met the inclusion criteria and were included in the final synthesis. These studies focused on the application of CRISPR technologies in various medicinal plants for improving yield, bioactive compound production, and stress resistance.

Distribution of Medicinal Plant Species

The 51 selected studies represented a diverse range of medicinal plant species, with the most frequently cited being Artemisia annua, Withania somnifera, Salvia miltiorrhiza, Catharanthus roseus, and Papaver somniferum. Less commonly studied plants included Glycyrrhiza uralensis, Camptotheca acuminata, Panax ginseng, and Curcuma longa.

CRISPR Systems and Gene Editing Tools

The majority of studies (n = 43) employed the CRISPR/Cas9 system, while Cas12a (Cpf1) was used in 6 studies, and newer base editors were utilized in 2 studies. The editing strategies included:

- Gene knockouts to suppress negative regulators of metabolite biosynthesis.
- Gene knock-ins to introduce or enhance biosynthetic pathway genes.
- Transcriptional activation systems to upregulate desirable traits.

Delivery Methods

Agrobacterium-mediated transformation was the most commonly used method for delivering CRISPR constructs (n = 34), followed by PEG-mediated protoplast transfection (n = 10), and biolistic methods (n = 7). Studies on species with complex genomes like Panax ginseng often use dual-vector systems to increase editing efficiency.

Target Genes and Editing Objectives

Category	No. of Studies	Genes/Targets	Plant Species	Purpose/Effect
Enhancement of Bioactive Compound Yield	31	ADS	Artemisia annua	Increased artemisinin content
		WRKY1, SQS	Withania somnifera	Withanolide biosynthesis
		CYP76AH1	Salvia miltiorrhiza	Tanshinone production
Improvement of Stress Tolerance	12	DREB, NAC, MYB (TFs)	Glycyrrhiza uralensis, Ocimum sanctum	Improved drought and salt stress resistance
Resistance to Pathogens	8	MLO, PRR	Catharanthus roseus, Curcuma longa	Improved resistance to fungal and bacterial infections

Outcomes and Effectiveness

Across the studies, successful editing was confirmed using PCR, Sanger sequencing, and next-generation sequencing (NGS). Most studies reported a 20% to 60% increase in targeted metabolite levels, while some achieved twofold to fivefold enhancements in compound yield (e.g., withanolides, artemisinin, tanshinones). Additionally, 9 studies demonstrated improved plant biomass and growth rate following gene editing.

Editing efficiency ranged from 35% to 80%, depending on the CRISPR system and transformation method. Cas12a-based systems showed slightly higher specificity but were used less frequently.

Quality of Studies

Based on the JBI quality checklist, 45 out of 51 studies met all seven criteria, while the remaining 6 met at least five. Most studies provided clear objectives, reproducible methodologies, and statistically validated outcomes. Only a few lacked detailed reporting of editing confirmation or metabolite quantification techniques.

DISCUSSION

The findings of this review confirm that CRISPR-based genome editing holds significant potential in enhancing both the yield and quality of medicinal plants. The majority of included studies reported successful editing of genes directly involved in secondary metabolite biosynthesis, stress resistance, or growth regulation—core traits responsible for improving medicinal plant productivity.

These results corroborate previous studies. For instance, Bashir *et al*. (2021) proved editing the ADS gene in Artemisia annua using CRISPR/Cas9 increased artemisinin from content from previously reported levels by 2.3-fold, and this supports trends presented in our review. Likewise, Mehta *et al*. (2022) proved that editing WRKY transcription factors in Withania somnifera caused an increased in withanolide levels, while not decreasing plant vigor.

Stress tolerance was another key area of focus. Matching our conclusions from the review, Ghosh *et al*. (2020) found that DREB1A (the drought resistant enhancer gene) edited in Ocimum sanctum improved drought by increasing drought tolerance, which then supported further secondary metabolite production, from a stressor response. We support this trend; 12 out of the 23 studies review targeted transcription factors to improve abiotic stress tolerances.

Exceptional in numbers, but not topic, is plant's resistances to pathogens which demonstrated prospects for such developments. CRISPR-mediated knockout of susceptibility genes such as MLO, or pathogen pattern recognition receptors (PRRs) have increased the resistance of many plant species (Tariq *et al*., 2019). Our paper indicates eight studies endorsed the promise of such interventions for medicinal plants such as Curcuma longa and Catharanthus roseus.

Notably, the frequent application of Agrobacterium-mediated transformation in our 34 out of 51 studies corresponds to its long-standing bias towards stable integration within plant systems. However, newer delivery methods, such as transient expression or protoplast editing, are gaining popularity due to their minimal genomic disruption (Raza *et al*., 2021).

Despite these advancements, challenges remain. As noted by Datta and Sood (2023), the genetic complexity and polyploidy of many medicinal plants make genome annotation and target identification difficult. This complexity limits the broader application of CRISPR in under-researched medicinal species. Furthermore, editing efficiency remains variable and heavily dependent on species, tissue culture responsiveness, and vector design.

Moreover, regulatory issues continue to hinder the commercialization of CRISPR-edited medicinal plants. As discussed by Ma *et al*. (2022), while some countries (e.g., USA, Brazil) have relaxed regulations for non-transgenic gene-edited crops, many others maintain strict controls, which may delay the adoption of CRISPR-derived medicinal products.

Nonetheless, the clear trend in recent studies—reflected in the 51 articles reviewed here—is that CRISPR is transitioning from a proof-of-concept tool to a widely adopted platform in medicinal plant biotechnology. The increasing use of Cas12, base editors, and prime editing systems is likely to address current limitations and enable multiplexed gene editing for more complex trait enhancements.

CONCLUSION

This review highlights the transformative role of CRISPR-based genome editing in improving the yield, quality, and resilience of medicinal plants. The synthesis of 51 recent studies demonstrates that CRISPR/Cas systems, particularly Cas9, have been effectively applied to manipulate key biosynthetic, regulatory, and defense-related genes across a variety of medicinal plant species. Targeted gene knockouts and activations have resulted in enhanced production of critical phytochemicals such as artemisinin, withanolides, and tanshinones, along with improved stress tolerance and pathogen resistance.

The findings also highlight the increasing diversity in CRISPR delivery systems and the emergence of newer tools, such as Cas12 and base editors, which offer greater precision and reduced off-target effects. Nonetheless, the appropriate application of these technologies is constrained by several challenges, including genome complexity, sub-par transformation systems, and regulatory uncertainties, particularly in less well-characterized medicinal species.

These challenges notwithstanding, CRISPR is transforming medicinal plant biotechnology and not only does it provide a pathway to sustainable, efficient, and high-yielding production of phytopharmaceuticals, but also offers new opportunities for conservation and mass propagation of rare medicinal species. Accordingly, future work must conduct studies to develop standard operating procedures for genome editing in non-model medicinal crops, better transformation systems, and consider the biosafety and regulatory concerns.

In conclusion, CRISPR has potential to revolutionized the continued efforts to fulfil the world's medicinal demands via biotechnology-led plant improvements.

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