

Research Insight

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Development of Transgenic *Eucommia ulmoides* for Improved Industrial Traits

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Abstract This study explores the recent advancements in the development of transgenic *Eucommia ulmoides* aimed at improving its industrial traits, particularly focusing on rubber production, stress tolerance, and secondary metabolite enhancement. It synthesizes key findings on the genetic basis underlying these traits, facilitated by high-quality genome assemblies and detailed QTL analyses. These genetic insights have laid the foundation for precise gene editing, enabling the introduction of desirable traits such as enhanced rubber biosynthesis and improved drought and salt tolerance. Through a case study, the study highlights the successful overexpression of key genes involved in rubber biosynthesis, resulting in significant increases in yield and quality. This case study provides practical insights into the application of genetic engineering in *E. ulmoides*, demonstrating its potential to transform the industry by offering a sustainable alternative to traditional rubber sources. The study concludes with a discussion on the broader implications of these developments for the *E. ulmoides* industry and the ethical considerations associated with transgenic research.

Keywords *Eucommia ulmoides*; Transgenic plants; Rubber biosynthesis; Genetic engineering; Stress tolerance

1 Introduction

Eucommia ulmoides, commonly known as the hardy rubber tree, is the sole species of the family Eucommiaceae. This species is native to China (Xie et al., 2023) and has been recognized for its significant industrial and medicinal applications. Traditionally, *E. ulmoides* has been utilized in Chinese medicine for its therapeutic properties, including anti-inflammatory and anti-hypertensive effects (Wang et al., 2019; Li et al., 2020). Additionally, it is valued for its unique ability to produce trans-polyisoprene rubber, a high-molecular mass polymer that is an essential raw material in various industrial applications.

E. ulmoides is particularly notable for several key industrial traits. It is a source of natural rubber, which is synthesized through the methylerythritol-phosphate (MEP) pathway, distinguishing it from other rubber-producing species like *Hevea brasiliensis* that utilize the mevalonate pathway (Li et al., 2020). This rubber, known as Eu-Rubber, is used in the production of various goods, including medical supplies and industrial products (Wang et al., 2017). Additionally, the species has significant medicinal properties, with compounds such as chlorogenic acid being preferentially expressed in its leaves, contributing to its therapeutic uses. The wood of *E. ulmoides* is also valued for its quality, making it a multifaceted resource for various industries.

Despite its valuable traits, the natural variation in *E. ulmoides* poses challenges for its industrial exploitation. Traditional breeding techniques are limited by the dioecious nature of the species, which complicates sex identification and breeding processes (Wang et al., 2020). Moreover, the genetic basis of key traits such as rubber biosynthesis and sex determination is not fully understood, hindering efforts to enhance these traits through conventional methods (Zhang et al., 2023).

This study is to explore the development of transgenic *E. ulmoides* for improved industrial traits. By leveraging recent advancements in genomic technologies and molecular biology, this study aims to identify key genetic factors involved in rubber biosynthesis, sex determination, and other industrially relevant traits. The expectation is that through genetic engineering, it will be possible to overcome the limitations of traditional breeding, thereby

enhancing the industrial and medicinal value of *E. ulmoides*, and providing insights into future directions for the genetic improvement of this economically important species.

2 Current State of *Eucommia ulmoides* Research

2.1 Summary of available genomic information and tools for *E. ulmoides*

Eucommia ulmoides, as a tree species with significant economic value, the research progress on its whole genome information is crucial for its genetic improvement. A comprehensive genetic linkage map has been developed using various molecular markers, including sequence-related amplified polymorphism, amplified fragment length polymorphism, inter-simple sequence repeat, and simple sequence repeat markers. This map encompasses 706 markers distributed across 25 linkage groups, covering approximately 89% of the estimated *E. ulmoides* genome with an average marker interval of 3.1 cM. This genetic linkage map is instrumental for identifying quantitative trait loci (QTL) associated with growth-related traits, thereby facilitating marker-assisted selection and further genomic studies in *E. ulmoides* (Li et al., 2014).

2.2 Traditional breeding efforts: overview of the breeding programs and their limitations

Traditional breeding programs for *Eucommia ulmoides* have primarily focused on improving phenotypic traits to meet the increasing demand for this versatile species. These programs have involved the selection of preferable genotypes and cultivation areas based on the significant effects of genotype, site, and genotype $\times$ environment interactions on phenotypic traits. However, the site effect has been found to account for a larger proportion of the variance in most traits (Deng et al., 2021), followed by genotype and genotype $\times$ environment interactions. This indicates that while traditional breeding can improve traits by selecting optimal genotypes or cultivation areas, it is limited by the significant environmental influence on trait performance. Additionally, the need for trait performance stability and the discriminating ability of genotypes in different cultivation areas further complicates traditional breeding efforts (Deng et al., 2022).

2.3 Early transgenic efforts

Initial attempts to genetically modify *Eucommia ulmoides* have been relatively limited compared to traditional breeding efforts. Early transgenic research has focused on understanding the genetic basis of key traits and developing tools for genetic manipulation. The construction of a genetic linkage map and the identification of QTLs for growth-related traits represent foundational steps towards more advanced genetic modification techniques. These efforts aim to overcome the limitations of traditional breeding by enabling more precise and targeted improvements in *E. ulmoides* traits. However, detailed reports on successful transgenic modifications and their outcomes are still sparse, indicating the nascent stage of transgenic research in this species (Li et al., 2014).

3 Target Traits for Industrial Improvement

3.1 Rubber production: genetic targets for increasing rubber yield and quality

Eucommia ulmoides, known for its production of trans-1,4-polyisoprene (Eu-rubber), has been the focus of several studies aiming to enhance rubber yield and quality through genetic modifications. The high-quality haploid genome assembly of *E. ulmoides* has provided significant insights into the rubber biosynthesis pathway, particularly the methylerythritol-phosphate (MEP) pathway, which is predominant in this species (Li et al., 2020). Additionally, the identification of long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) involved in rubber biosynthesis has opened new avenues for genetic engineering. Specific lncRNAs and miRNAs have been found to regulate key genes in the rubber biosynthesis pathway, suggesting potential targets for increasing rubber yield. For instance, after processing the assembled transcripts through a rigorous procedure, Liu et al. (2018a) identified 29 103 lncRNAs (Figure 1) and found that these lncRNAs are involved in 12 protein-coding genes as well as 95 DE genes related to Eu-rubber biosynthesis. Moreover, the application of growth regulators has been shown to significantly enhance TPI productivity, indicating that both genetic and agronomic approaches can be combined for optimal results (Liu et al., 2018b).

3.2 Disease resistance: enhancing resistance to pests and diseases

Improving disease resistance in *E. ulmoides* is crucial for maintaining healthy plantations and ensuring consistent rubber production. The high-quality genome assembly of *E. ulmoides* has identified numerous protein-coding

genes that could be targeted for enhancing disease resistance. Additionally, the role of miRNAs in stress response processes has been highlighted, with several miRNAs identified as potential regulators of genes involved in disease resistance (Wang et al., 2016; Ye et al., 2019). By leveraging these genetic insights, it is possible to develop transgenic *E. ulmoides* varieties with enhanced resistance to pests and diseases, thereby reducing the reliance on chemical pesticides and improving overall plantation health.

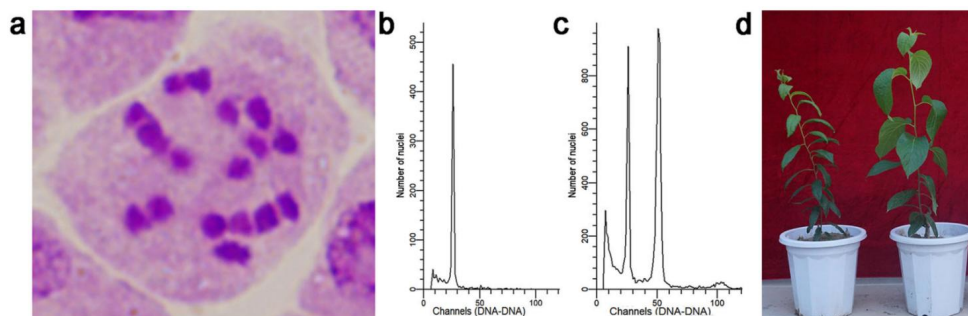


Figure 1 a) Somatic chromosome number of the haploids ($2n=x=17$); b) Ploidy levels obtained from 3-week-old first leaf samples from haploid plants by flow cytometric analysis; c) Ploidy levels obtained from 3-week-old first leaf samples from a mixture of haploid and diploid plants by flow cytometric analysis; d) A haploid plant (left) and diploid plant (right) of *E. ulmoides* (Adopted from Li et al., 2020)

3.3 Growth and biomass: modifications aimed at increasing growth rate and biomass production

Increasing the growth rate and biomass production of *E. ulmoides* is essential for maximizing rubber yield and other industrial applications. Studies have shown that the application of growth regulators can significantly enhance biomass yield, tree height, and leaf size, which are directly correlated with increased rubber production. Researchers have utilized a high temperature-induced spore chromosome doubling method to breed new triploid varieties of *Eucommia ulmoides* with significant advantages such as rapid growth, gigantic leaves, and high content of effective components. These triploid *Eucommia* varieties have demonstrated the potential to provide excellent and low-cost raw materials for the development of rubber industry, pharmaceutical industry, and feed additive industry (Li et al., 2016). Furthermore, the phenotypic trait analysis of different *E. ulmoides* clones has revealed that both genotype and environmental factors play significant roles in growth traits, suggesting that selective breeding and optimized cultivation practices can further improve growth and biomass production (Deng et al., 2022). These findings provide a comprehensive framework for developing high-yielding *E. ulmoides* varieties through both genetic and agronomic interventions.

3.4 Secondary metabolites: improvement of medicinal and other valuable compounds

Eucommia ulmoides is a valuable medicinal plant containing various bioactive secondary metabolites like iridoids, lignans, phenolics, and terpenoids. These compounds have demonstrated anti-oxidative, anti-inflammatory, anti-cancer, and other pharmacological activities beneficial for human health. *E. ulmoides* is not only valued for its rubber production but also for its medicinal properties, primarily due to the presence of secondary metabolites such as chlorogenic acid. The high-quality genome assembly has identified key enzymes involved in the chlorogenic acid biosynthesis pathway, which are predominantly expressed in the leaves. This provides a genetic basis for enhancing the production of chlorogenic acid and other valuable secondary metabolites through targeted genetic modifications. Meanwhile, studies have shown that treatment with different growth regulators and trace elements can significantly increase the content of secondary metabolites in *Eucommia ulmoides*. Additionally, the phenotypic trait analysis has shown that environmental factors such as annual mean temperature and sunshine duration significantly affect the content of secondary metabolites, indicating that optimized cultivation practices can also play a crucial role in improving the medicinal value of *E. ulmoides* (Deng et al., 2022).

4 Molecular Tools and Techniques Used in Transgenic Development

4.1 Gene editing technologies

Gene editing technologies have revolutionized the field of plant biotechnology, enabling precise modifications to the genome of *Eucommia ulmoides* to enhance its industrial traits. One of the key advancements in this area is the

acquisition of a high-quality haploid chromosome-scale genome assembly for *E. ulmoides*. This assembly, achieved using PacBio and Hi-C technologies, provides a robust foundation for gene editing by offering detailed insights into the genome structure and evolution, which are crucial for targeted genetic modifications (Li et al., 2020). Additionally, the identification of 26 001 predicted protein-coding genes anchored to the 17 chromosomes facilitates the precise editing of genes involved in rubber biosynthesis and other industrially relevant pathways.

4.2 Transformation methods

Transformation methods are essential for introducing new genetic material into *E. ulmoides*. One widely used method is *Agrobacterium*-mediated transformation, which has been successfully employed to obtain transgenic lines of *Arabidopsis thaliana* expressing *Eucommia ulmoides* genes. For instance, the promoter sequences of the *Eucommia ulmoides* SRPP (EuSRPP) genes were introduced into *Arabidopsis* via *Agrobacterium*, resulting in stable expression in various plant tissues (Zhao et al., 2023). Furthermore, researchers have established a protoplast isolation and transient transformation system for *Eucommia ulmoides*, laying the foundation for high-throughput analysis of gene function studies in the future (Hu et al., 2024). Simultaneously, there has been some research on the method of using *Agrobacterium* infection of the hypocotyl of *Eucommia ulmoides* to induce new plantlets (Ran et al., 2022), this method not only demonstrates the feasibility of genetic transformation in *E. ulmoides* but also highlights the potential for using similar techniques to enhance the rubber biosynthesis pathway and other desirable traits in this species.

4.3 Promoters and regulatory elements

Promoters and regulatory elements play a critical role in the expression of transgenes in *E. ulmoides*. The activity of these elements can be influenced by various environmental and endogenous factors. For example, the promoter activity of the *Eucommia ulmoides* SRPP genes was found to be regulated by methyl jasmonate (MeJA), gibberellin (GA3), and drought pathways. The expression activity of these promoters varied under different treatments, indicating that they are responsive to hormonal and stress signals (Zhao et al., 2023). This knowledge is invaluable for designing transgenic constructs with promoters that can drive the expression of target genes in a controlled manner, thereby optimizing the production of industrially important compounds in *E. ulmoides*.

5 Case Study: Successful Development of Transgenic *Eucommia ulmoides* for Enhanced Rubber Production

5.1 Methodology and results

The development of transgenic *Eucommia ulmoides* aimed at enhancing rubber production has been a significant focus in recent years. *Eucommia ulmoides*, known for its production of trans-polyisoprene rubber, presents a valuable alternative to traditional rubber sources.

The primary approach involved the overexpression of key genes involved in the rubber biosynthesis pathway. One notable project isolated and overexpressed the isopentenyl diphosphate isomerase (IPI) gene in *Eucommia ulmoides*. This gene plays a crucial role in the conversion of isopentenyl diphosphate to dimethylallyl diphosphate, a precursor in the biosynthesis of trans-polyisoprene (Chen et al., 2012). The transformation was achieved using *Agrobacterium*-mediated techniques, which allowed for the stable integration and expression of the transgene in the plant genome.

The transgenic lines of *Eucommia ulmoides* exhibited significant improvements in rubber production. Specifically, the overexpression of the EuIPI gene resulted in a 3- to 4-fold increase in the total content of trans-polyisoprenes compared to non-transgenic controls (Chen et al., 2012). Additionally, transcriptome analysis identified several differentially expressed genes involved in terpenoid biosynthesis, further supporting the enhanced rubber production in transgenic lines (Jin et al., 2020). Li et al. (2020) obtained three authentic *Eucommia* haploid plants (Figure 1) through flow cytometry and analysis of anatomical sections of stem tips, and performed genome sequencing and assembly. The successful assembly of a high-quality haploid genome also provided insights into the genetic basis of rubber biosynthesis, facilitating further genetic improvements.

5.2 Impact and challenges

The development of transgenic *Eucommia ulmoides* has significant implications for the rubber industry. The enhanced production of trans-polyisoprene provides a sustainable and economically viable alternative to traditional rubber sources. The genetic insights gained from these studies also pave the way for further improvements in rubber yield and quality. Moreover, the successful application of genetic engineering techniques in *Eucommia ulmoides* serves as a model for similar efforts in other economically important plant species (Jin et al., 2020).

One of the primary challenges in developing transgenic *Eucommia ulmoides* was the low yield of rubber in natural conditions. To address this, researchers focused on the molecular regulation of key biosynthetic genes. For instance, the promoter activity of the small rubber particle protein (SRPP) gene was analyzed, revealing that its expression is regulated by endogenous hormones and environmental stressors such as drought (Zhao et al., 2023). This understanding allowed for the optimization of gene expression under various conditions, thereby enhancing rubber yield. Additionally, heterologous co-expression of the key rubber biosynthetic genes *EuTPT5* and *EuREF1* in *Nicotiana benthamiana* did not result in a significant increase in rubber yield and molecular weight as expected. The biosynthesis of natural rubber is likely a complex process involving multiple enzymes. Therefore, further exploration of rubber biosynthesis may need to be conducted within *Eucommia ulmoides* itself.

6 Challenges in Developing Transgenic *Eucommia ulmoides*

6.1 Difficulties in gene transformation and regeneration of transgenic plants

Developing transgenic *Eucommia ulmoides* presents significant technical challenges, particularly in the areas of gene transformation and plant regeneration. The complexity of the *E. ulmoides* genome, as highlighted by the high-quality de novo assembly, underscores the difficulty in achieving efficient gene transformation and stable integration of transgenes (Li et al., 2020). Additionally, the intricate process of regenerating transgenic plants from transformed cells is compounded by the species-specific requirements for tissue culture and regeneration protocols, which are not yet fully optimized for *E. ulmoides* (Li et al., 2014; Jin et al., 2020).

6.2 Issues related to the stability of transgenes across generations

Ensuring the genetic stability of transgenes across multiple generations is another critical challenge. The stability of transgenes can be influenced by various factors, including the genomic context of the insertion site and the potential for gene silencing. Studies on the genetic linkage map and QTL analysis for *E. ulmoides* have revealed significant genetic variability and complex inheritance patterns, which could affect the stable expression of transgenes (Jin et al., 2020; Liu et al., 2022). Moreover, the presence of mutation hotspots and heterogeneous sequence divergence in the chloroplast genome further complicates the maintenance of genetic stability in transgenic lines.

6.3 Challenges posed by regulatory frameworks and environmental impact assessments

The development and deployment of transgenic *E. ulmoides* are subject to stringent regulatory frameworks and environmental impact assessments. Regulatory bodies require comprehensive data on the safety and environmental impact of transgenic plants, including potential effects on non-target organisms and gene flow to wild relatives. The unique ecological and economic importance of *E. ulmoides*, as well as its status as a Tertiary relic plant endemic to China, necessitates thorough environmental assessments to ensure that transgenic varieties do not adversely affect biodiversity or ecosystem stability (Wang et al., 2018; Deng et al., 2022). Additionally, the regulatory approval process can be lengthy and resource-intensive, posing a significant barrier to the commercialization of transgenic *E. ulmoides* (Chen et al., 2022a; Chen et al., 2022b).

7 Field Trials and Commercialization Prospects

7.1 Field trial results

Field trials are essential for evaluating the performance and environmental impact of transgenic *Eucommia ulmoides*. Recent studies have shown that the phenotypic traits of *E. ulmoides* can be significantly influenced by both genotype and environmental factors. For instance, a study involving nine clones across six different sites demonstrated that site effects accounted for a larger proportion of variance in most traits, followed by genotype

and genotype $\times$ environment interactions. This indicates that selecting optimal genotypes and cultivation areas can substantially improve both growth and economic traits of *E. ulmoides*. Additionally, the updated genetic linkage map and QTL analysis for growth traits over a decade have identified several quantitative trait loci (QTLs) that are crucial for tree height, ground diameter, and crown diameter, providing a genetic basis for trait improvement (Jin et al., 2020).

7.2 Commercial viability

The commercial viability of transgenic *E. ulmoides* hinges on its improved industrial traits, such as enhanced rubber biosynthesis and medicinal properties. The high-quality de novo assembly of the *E. ulmoides* genome has provided new insights into its rubber biosynthesis pathways, which differ from those in *Hevea brasiliensis*. This genomic information can be leveraged to genetically engineer *E. ulmoides* for better industrial applications, making it a more attractive option for commercial cultivation (Li et al., 2020). Furthermore, enhancing the production of these high-value secondary metabolites through approaches like elicitation with growth regulators or trace element supplementation is an important area of research to improve the medicinal and economic value of *E. ulmoides* cultivation. Optimizing the accumulation of key bioactive metabolites can augment the therapeutic potential and commercial viability of this species. Specifically, the ability to improve both growth and economic traits simultaneously through careful selection of genotypes and cultivation areas enhances the commercial prospects of this species (Deng et al., 2022).

7.3 Regulatory approvals

Regulatory approval is a critical step for the commercialization of transgenic crops, including *E. ulmoides*. The regulatory landscape varies significantly across regions, with the European Union (EU) known for its stringent regulations. Field trials in the EU must demonstrate that the risk associated with transgenic crops is no greater than that of conventional crops. This involves multiple layers of bureaucracy and adherence to the precautionary approach, which assumes inherent risks in all transgenic crops. Strategies to streamline these regulations without adding unnecessary burdens on developers are essential for the efficient commercialization of transgenic *E. ulmoides* (Gómez-Galera et al., 2012).

8 Environmental and Ecological Impacts

8.1 Risk assessment: analysis of potential ecological risks associated with transgenic *E. ulmoides*

The introduction of transgenic *Eucommia ulmoides* (*E. ulmoides*) into the environment necessitates a thorough risk assessment to evaluate potential ecological impacts. One of the primary concerns is the possibility of gene flow from transgenic *E. ulmoides* to wild relatives, which could lead to unintended ecological consequences. Studies have shown that the genetic modification of *E. ulmoides*, such as the overexpression of aquaporin genes like *EuPIP1;2* and *EuPIP1;1*, can enhance drought and salt tolerance in transgenic plants (Chen et al., 2022b). While these traits are beneficial for cultivation, they may also confer a competitive advantage to transgenic plants in natural ecosystems, potentially disrupting local biodiversity. Additionally, the long-term stability and expression of these transgenes in various environmental conditions need to be monitored to understand their ecological impact fully.

8.2 Biosafety measures: strategies to mitigate risks and ensure safe cultivation

To mitigate the potential ecological risks associated with transgenic *E. ulmoides*, several biosafety measures should be implemented. For example, the use of genetic containment strategies, such as male sterility or seed sterility, can prevent the spread of transgenes to wild populations. Additionally, spatial isolation of transgenic *E. ulmoides* plantations from natural habitats can reduce the risk of gene flow. Regular monitoring and environmental impact assessments should be conducted to detect any unintended effects on non-target organisms and ecosystems (Ghimire et al., 2023; Eckerstorfer et al., 2023). Furthermore, the development of a comprehensive regulatory framework that includes guidelines for the safe cultivation, handling, and disposal of transgenic *E. ulmoides* is essential to ensure biosafety (Jin et al., 2020; Deng et al., 2022; Basha and Kader, 2022).

8.3 Long-term impact: consideration of the long-term effects on biodiversity and ecosystems

The long-term environmental impact of transgenic *E. ulmoides* on biodiversity and ecosystems requires careful consideration. The introduction of transgenic plants with enhanced traits, such as improved growth and stress tolerance, could alter the dynamics of local plant communities and affect the associated fauna. For instance, the overexpression of aquaporin genes in *E. ulmoides* has been shown to improve plant growth and stress tolerance, which could lead to changes in plant competition and resource allocation in natural ecosystems (Chen et al., 2022a).

Additionally, the potential accumulation of transgene products in the soil and their effects on soil microbiota and nutrient cycling need to be investigated (Powell et al., 2009; Liu, 2010). Long-term field studies and ecological modeling can provide valuable insights into the potential impacts of transgenic *E. ulmoides* on ecosystem functions and services. It is crucial to adopt a precautionary approach and continuously evaluate the environmental consequences of transgenic *E. ulmoides* to ensure the sustainability of both agricultural and natural ecosystems (Li et al., 2014).

9 Future Directions in Transgenic *Eucommia ulmoides* Research

9.1 Emerging technologies

The development of new genetic engineering tools such as CRISPR/Cas9 and other genome-editing technologies holds significant promise for advancing transgenic research in *Eucommia ulmoides*. These tools can facilitate precise modifications in the genome, enabling the introduction of desirable traits such as enhanced drought and salt tolerance. For instance, the overexpression of aquaporin genes like *EuPIP1;2* has already demonstrated improved stress tolerance in transgenic tobacco, suggesting similar applications could be beneficial in *E. ulmoides* (Chen et al., 2022b). Additionally, the high-quality de novo assembly of the *E. ulmoides* genome provides a robust framework for identifying target genes and understanding their functions, which can be leveraged for more effective genetic modifications (Jain, 2015; Li et al., 2020). For example, taking the *EuFLC* (Flowering Locus C) gene as the target, a CRISPR/Cas9 gene editing system for *Eucommia ulmoides* has been established.

9.2 Integrative approach

Integrating traditional breeding methods with modern biotechnological approaches can significantly enhance the genetic improvement of *E. ulmoides*. Molecular marker technologies, such as those used in updating the genetic linkage map and QTL analysis for growth traits, can elucidate the genetic mechanisms underlying important traits and improve breeding efficiency (Jin et al., 2020). By combining these insights with genetic engineering techniques, it is possible to accelerate the development of *E. ulmoides* varieties with superior industrial traits. For example, the overexpression of aquaporin genes like *EuPIP1;1* in *Arabidopsis* has shown to promote growth and stress tolerance, indicating potential pathways for similar enhancements in *E. ulmoides*.

9.3 Sustainable development goals

Aligning the development of transgenic *E. ulmoides* with global sustainability initiatives is crucial for ensuring that biotechnological advancements contribute to broader environmental and social goals. The enhanced stress tolerance conferred by genes such as *EuPIP1;2* and *EuPIP1;1* can lead to more resilient crops that require fewer resources, thereby supporting sustainable agricultural practices (Chen et al., 2022a). Furthermore, the high-quality genome assembly of *E. ulmoides* can aid in the development of varieties that are not only more productive but also more environmentally friendly, by optimizing rubber biosynthesis pathways and reducing reliance on traditional rubber sources. These efforts can contribute to achieving Sustainable Development Goals (SDGs) related to responsible consumption and production, climate action, and life on land (Wuyun et al., 2017).

10 Concluding Remarks

This study explores the major advancements in transgenic *Eucommia ulmoides* research in recent years. Gene editing technologies have provided powerful tools for the precise modification of the *Eucommia ulmoides* genome, making it possible to introduce key industrial traits such as enhanced rubber biosynthesis, drought tolerance, and salt tolerance. Additionally, high-quality genome assembly and related genetic linkage maps and QTL analyses have revealed the genetic basis associated with growth, economic traits, and secondary metabolites, offering

crucial support for future genetic improvements. Through these studies, transgenic *E. ulmoides* has shown great potential in increasing rubber yield, enhancing stress resistance, and optimizing the production of secondary metabolites.

These developments have profound implications for the future of the *Eucommia ulmoides* industry. With further optimization of transgenic technologies, *E. ulmoides* is expected to significantly strengthen its position as a sustainable alternative to natural rubber. Furthermore, the medicinal value and production of other secondary metabolites in *E. ulmoides* will also be enhanced through these genetic engineering technologies, expanding its applications in the pharmaceutical and related industries. These advancements not only have the potential to improve the economic benefits of *E. ulmoides* but also reduce reliance on traditional rubber sources, promoting more environmentally friendly and sustainable industrial practices.

Looking forward, the development potential of transgenic *E. ulmoides* is vast. However, as these technologies mature, ethical and ecological considerations must also be addressed. The long-term environmental impact of transgenic plants needs to be continuously monitored to ensure they do not negatively affect natural ecosystems. Additionally, as the *E. ulmoides* industry expands, it is necessary to establish clear regulatory frameworks to balance technological progress with social responsibility. Overall, transgenic *E. ulmoides* opens new horizons for rubber production and other industrial applications, but these advancements must be aligned with global sustainability goals to ensure their long-term environmental and economic benefits.

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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

- Basha N., and kader A., 2022, Genetically modified crops, production, detection methods and its biosafety implications: a scientific review, Arab Journal for Plant Protection. 40: 260-279.
<https://doi.org/10.22268/AJPP-40.3.260279>
- Chen J., Huang Y., Li J., Li Y., Zeng X., and Zhao D., 2022a, Overexpression of the *Eucommia ulmoides* aquaporin, *EuPIP1;1*, promotes leaf growth, flowering and bolting, and stress tolerance in Arabidopsis, International Journal of Molecular Sciences, 23(19): 11794.
<https://doi.org/10.3390/ijms231911794>
PMid:36233096 PMCID:PMC9570377
- Chen J., Li J., Huang Y., Li Y., Su C., and Zeng X., 2022b, *EuPIP1;2*, a plasma membrane aquaporin gene from *Eucommia ulmoides*, enhances drought and salt tolerance in transgenic tobacco, Agronomy, 12(3): 615.
<https://doi.org/10.3390/agronomy12030615>
- Chen R., Harada Y., Bamba T., Nakazawa Y., and Gyokusen K., 2012, Overexpression of an isopentenyl diphosphate isomerase gene to enhance *trans*-polyisoprene production in *Eucommia ulmoides* Oliver, BMC Biotechnology, 12: 78.
<https://doi.org/10.1186/1472-6750-12-78>
PMid:23110380 PMCID:PMC3547716
- Deng P., Wang Y., Hu F., Yu H., Liang Y., Zhang H., Wang T., Zhou Y., and Li Z., 2022, Phenotypic trait subdivision provides new sight into the directional improvement of *Eucommia ulmoides* Oliver, Frontiers in Plant Science, 13: 832821.
<https://doi.org/10.3389/fpls.2022.832821>
PMid:35463430 PMCID:PMC9026163

- Deng P., Xie X., Long F., Zhang L., Li Y., Zhao Z., Yang S., Wang Y., Fan R., and Li Z., 2021, Trait variations and probability grading index system on leaf-related traits of *Eucommia ulmoides* Oliver germplasm, *Plants*, 10(11): 2280.
<https://doi.org/10.3390/plants10112280>
 PMid:34834643 PMCID:PMC8620490
- Eckerstorfer M., Dolezel M., Engelhard M., Giovannelli V., Grabowski M., Heissenberger A., Lener M., Reichenbecher W., Simon S., Staiano G., Saucy A., Zünd J., and Lüthi C., 2023, Recommendations for the assessment of potential environmental effects of genome-editing applications in plants in the EU, *Plants*, 12(9): 1764.
<https://doi.org/10.3390/plants12091764>
 PMid:37176822 PMCID:PMC10180588
- Ghimire B., Yu C., Kim W., Moon H., Lee J., Kim S., and Chung I., 2023, Assessment of benefits and risk of genetically modified plants and products: current controversies and perspective, *Sustainability*, 15(2): 1722.
<https://doi.org/10.3390/su15021722>
- Gómez-Galera S., Twyman R., Sparrow P., Droogenbroeck B., Custers R., Capell T., and Christou P., 2012, Field trials and tribulations-making sense of the regulations for experimental field trials of transgenic crops in Europe, *Plant Biotechnology Journal*, 10(5): 511-523.
<https://doi.org/10.1111/j.1467-7652.2012.00681.x>
 PMid:22284604
- Hu B., Dong M., Liu R., Shan W., Wang Y., Ding Y., Peng J., Meng L., Wang C., and Zhou Q., 2024, Establishment of an efficient protoplast isolation and transfection method for *Eucommia ulmoides* Oliver, *Frontiers in Bioscience (Landmark edition)*, 29(5): 187.
<https://doi.org/10.31083/j.fbl2905187>
 PMid:38812327
- Jain M., 2015, Function genomics of abiotic stress tolerance in plants: a CRISPR approach, *Frontiers in Plant Science*, 6: 375.
<https://doi.org/10.3389/fpls.2015.00375>
- Jin C., Li Z., Li Y., Wang S., Li L., and Liu M., 2020, Update of genetic linkage map and QTL analysis for growth traits in *Eucommia ulmoides* Oliver, *Forests*, 11(3): 311.
<https://doi.org/10.3390/f11030311>
- Jin C., Li Z., Li Y., Wang S., Li L., Liu M., and Ye J., 2020, Transcriptome analysis of terpenoid biosynthetic genes and simple sequence repeat marker screening in *Eucommia ulmoides*, *Molecular Biology Reports*, 47: 1979-1990.
<https://doi.org/10.1007/s11033-020-05294-w>
 PMid:32040708
- Li W., Zhang J., Xie X., and Shi X., 2024, Research progress on chemical components, pharmacological activities and modern applications of *Eucommiae Folium* in Chinese, *Natural Product Research and Development*, 36(5): 900-917.
- Li Y., Wang D., Li Z., Wei J., Jin C., and Liu M., 2014, A molecular genetic linkage map of *Eucommia ulmoides* and quantitative trait loci (QTL) analysis for growth traits, *International Journal of Molecular Sciences*, 15(2): 2053-2074.
<https://doi.org/10.3390/ijms15022053>
 PMid:24477264 PMCID:PMC3958837
- Li Y., Wei H., Yang J., Du K., Li J., Zhang Y., Qiu T., Liu Z., Ren Y., Song L., and Kang X., 2020, High-quality de novo assembly of the *Eucommia ulmoides* haploid genome provides new insights into evolution and rubber biosynthesis, *Horticulture Research*, 7(1): 183.
<https://doi.org/10.1038/s41438-020-00406-w>
 PMid:33328448 PMCID:PMC7603500
- Liu C., Wang L., Lu W., Zhong J., Du H., Liu P., Du Q., Du L., and Qing J., 2022, Construction of SNP-based high-density genetic map using genotyping by sequencing (GBS) and QTL analysis of growth traits in *Eucommia ulmoides* Oliver, *Forests*, 13(9): 1479.
<https://doi.org/10.3390/f13091479>
- Liu H., Lu Y., Wang J., Hu J., and Wuyun T., 2018a, Genome-wide screening of long non-coding RNAs involved in rubber biosynthesis in *Eucommia ulmoides*, *Journal of Integrative Plant Biology*, 60(11): 1070-1082.
<https://doi.org/10.1111/jipb.12693>
 PMid:29944209
- Liu H., Zhu J., Ding H., Wang L., Sun Z., Liu P., Li T., and Du H., 2018b, Foliar spray of growth regulators significantly increases trans-1,4-polyisoprene production from *Eucommia ulmoides* Oliver short-rotation coppice, *Industrial Crops and Products*, 113113(2018): 383-390.
<https://doi.org/10.1016/j.indcrop.2018.01.054>
- Liu W., 2010, Do genetically modified plants impact arbuscular mycorrhizal fungi, *Ecotoxicology*, 19(2): 229-238.
<https://doi.org/10.1007/s10646-009-0423-1>
 PMid:19806453
- Powell J., Levy-Booth D., Gulden R., Asbil W., Campbell R., Dunfield K., Hamill A., Hart M., Lerat S., Nurse R., Pauls K., Sikkema P., Swanton C., Trevors J., and Klironomos J., 2009, Effects of genetically modified, herbicide-tolerant crops and their management on soil food web properties and crop litter decomposition, *Journal of Applied Ecology*, 46(2): 388-396.
<https://doi.org/10.1111/j.1365-2664.2009.01617.x>

- Ran X., Liu Y., and Zhao D., 2022, The relationship between *EuSRPP1* gene expression and rubber biosynthesis in *Eucommia ulmoides* Oliver (Du-zhong), Industrial Crops and Products, 175(2022): 114246.
<https://doi.org/10.1016/j.indcrop.2021.114246>
- Wang C., Gong H., Feng M., and Tian C., 2023, Phenotypic variation in leaf, fruit and seed traits in natural populations of *Eucommia ulmoides*, a relict Chinese endemic tree, Forests, 14(3): 462.
<https://doi.org/10.3390/f14030462>
- Wang C., Tang L., He J., Li J., and Wang Y., 2019, Ethnobotany, phytochemistry and pharmacological properties of *Eucommia ulmoides*: a review, The American Journal of Chinese Medicine, 47(2): 259-300.
<https://doi.org/10.1142/S0192415X19500137>.
PMid:30857406
- Wang L., Du H., and Wuyun T., 2016, Genome-wide identification of microRNAs and their targets in the leaves and fruits of *Eucommia ulmoides* using high-throughput sequencing, Frontiers in Plant Science, 7: 1632.
<https://doi.org/10.3389/fpls.2016.01632>
- Wang L., Jing T., Li T., Du H., and Wuyun T., 2017, Identification and expression analysis of the *Eucommia ulmoides* farnesyl diphosphate synthase gene family to reveal the key gene involved in rubber biosynthesis, Acta Physiologiae Plantarum, 40: 11.
<https://doi.org/10.1007/s11738-017-2588-1>
- Wang W., Chen S., and Zhang X., 2018, Whole-genome comparison reveals heterogeneous divergence and mutation hotspots in chloroplast genome of *Eucommia ulmoides* Oliver, International Journal of Molecular Sciences, 19(4): 1037.
<https://doi.org/10.3390/ijms19041037>
PMid:29601491 PMCID:PMC5979487
- Wang W., Yang G., Deng X., Shao F., Li Y., Guo W., Liang H., and Zhang X., 2020, Molecular sex identification in the hardy rubber tree (*Eucommia ulmoides* Oliver) via ddRAD markers, International Journal of Genomics, 2020(1): 2420976.
<https://doi.org/10.1155/2020/2420976>
PMid:32509842 PMCID:PMC7246395
- Wuyun T., Wang L., Liu H., Wang X., Zhang L., Bennetzen J., Li T., Yang L., Liu P., Du L., Wang L., Huang M., Qing J., Zhu L., Bao W., Li H., Du Q., Zhu J., Yang H., Yang S., Liu H., Yue H., Hu J., Yu G., Tian Y., Liang F., Hu J., Wang D., Gao R., Li D., and Du H., 2017, The hardy rubber tree genome provides insights into the evolution of polyisoprene biosynthesis, Molecular Plant, 11(3): 429-442.
<https://doi.org/10.1016/j.molp.2017.11.014>
PMid:29229569
- Xie S., Si H., Sun H., Zhao Q., Li X., Wang S., Niu J., and Wang Z., 2023, Predicting the potential distribution of the endangered plant *Eucommia ulmoides* in China under the background of climate change, Sustainability. 15(6): 5349.
<https://doi.org/10.3390/su15065349>
- Ye J., Han W., Fan R., Liu M., Li L., and Jia X., 2019, Integration of transcriptomes, small RNAs, and degradome sequencing to identify putative miRNAs and their targets related to Eu-rubber biosynthesis in *Eucommia ulmoides*, Genes, 10(8): 623.
<https://doi.org/10.3390/genes10080623>
PMid:31430866 PMCID:PMC6722833
- Zhang X., Wang X., Pan L., Guo W., Li Y., and Wang W., 2023, Genome-wide identification and expression analysis of MADS-box transcription factors reveal their involvement in sex determination of hardy rubber tree (*Eucommia ulmoides* Oliv.), Frontiers in Genetics, 14: 1138703.
<https://doi.org/10.3389/fgene.2023.1138703>
PMid:36896236 PMCID:PMC9988917
- Zhao H., Yao X., and Lu L., 2023, The amplification and activity analysis of the small rubber particle protein (SRPP) promoter of *Eucommia ulmoides* (EuSRPP) revealed that its activity was regulated by MeJA, GA3, and drought pathways, Horticulturae, 9(8): 856.
<https://doi.org/10.3390/horticulturae9080856>

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