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Evolutionary Pathways of Rubber Biosynthesis in *Eucommia ulmoides*

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Abstract *Eucommia ulmoides*, a relict tree species from the Tertiary Period. It was commonly known as the hardy rubber tree, has garnered significant attention due to its unique ability to produce trans-polyisoprene, a type of natural rubber， also known as Eucommia rubber,. This study delves into the evolutionary pathways of rubber biosynthesis in *Eucommia ulmoides*, highlighting the genetic, biochemical, and molecular mechanisms underlying this process. Based on the research findings of the rubber synthesis pathways of other latex-producing species such as Brazilian rubber tree (Hevea brasiliensis), the unique biosynthesis pathway of eucommia rubber is being continuously elucidated.. Notably, *Eucommia ulmoides* can utilize two pathways, both the methylerythritol phosphate (MEP) pathway and the methylvalerate (MVA) pathway, to synthesize the rubber synthesis precursor isopentenyl diphosphate (IPP). In the elongation process of eucommia rubber long-chain molecules,, the farnesyl diphosphate synthases (FPSs) ，Small rubber particle proteins (SRPPs) and rubber elongation factors (REFs) played the key roles. Additionally, the regulatory roles of long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) in rubber biosynthesis have been elucidated, offering potential targets for genetic engineering to enhance rubber yield. This study synthesizes current knowledge and identifies future research directions to further understand and exploit the rubber biosynthesis pathways in *Eucommia ulmoides* for industrial and medical applications.
Keywords Hardy rubber tree (*Eucommia ulmoides*); Trans-polyisoprene; Rubber biosynthesis; Methylerythritol-phosphate pathway; Mevalonate pathway; Farnesyl diphosphate synthase; Non-coding RNAs

1 Introduction

Eucommia ulmoides, commonly known as the Hardy Rubber Tree, is a significant plant species due to its unique ability to produce trans-polyisoprene rubber, also referred to as Eu-rubber. This tree is not only valued for its resilience and adaptability to various environmental conditions but also for its economic and industrial potential. Natural rubber is a critical raw material with extensive applications in industries ranging from automotive to healthcare. The global demand for natural rubber underscores its economic importance, making the study of alternative rubber-producing plants like *Eucommia ulmoides* highly relevant (Wuyun et al., 2017; Jin et al., 2020).

Unlike the more commonly known rubber tree, Hevea brasiliensis, which produces cis-polyisoprene, *Eucommia ulmoides* synthesizes trans-polyisoprene. This distinction places *Eucommia ulmoides* in a unique position within the rubber production industry, offering potential advantages in terms of material properties and applications. The biosynthesis of trans-polyisoprene in *Eucommia ulmoides* involves distinct genetic and biochemical pathways, which have evolved independently from those in *Hevea brasiliensis* (Wuyun et al., 2017; Wang et al., 2017). Understanding these pathways can provide insights into the evolutionary mechanisms of rubber biosynthesis and open up new avenues for biotechnological advancements in rubber production.

The primary goal of this study is to elucidate the evolutionary pathways of rubber biosynthesis in *Eucommia ulmoides*. By leveraging genomic, transcriptomic, and molecular analyses, this research aims to uncover the genetic and biochemical underpinnings of trans-polyisoprene production in this unique plant species. Specifically, the study seeks to address key questions regarding the genetic and molecular mechanisms underlying the biosynthesis of trans-polyisoprene in *Eucommia ulmoides*, and how these mechanisms have evolved compared to other rubber-producing plants, particularly Hevea brasiliensis. Additionally, the research will investigate the roles of specific genes, such as farnesyl diphosphate synthases (FPSs)， Small rubber particle proteins (SRPPs) and rubber elongation factors (REFs), in the regulation and synthesis of Eu-rubber. The insights gained from this study aim to enhance rubber production and improve the material properties of Eu-rubber, contributing to a deeper understanding of rubber biosynthesis in *Eucommia ulmoides* and exploring its potential applications in various industrial sectors.

2 Overview of *Eucommia ulmoides*

2.1 Taxonomy and distribution

Eucommia ulmoides belongs to the order Garryales and the Eucommiaceae family, and is a single species within its own genus.. It is a unique species within this order, as it is the first to have its genome sequenced, revealing significant insights into its evolutionary history and biological characteristics (Wuyun et al., 2017). *Eucommia ulmoides* is a sister taxon to lamiids and campanulids, and it has undergone an ancient genome triplication shared by core eudicots, but no further whole-genome duplication in the last approximately 125 million years (Wuyun et al., 2017). This species is native to China and is primarily distributed in temperate regions, where it has adapted to various environmental conditions due to its high expression levels and gene number expansion for multiple genes involved in stress responses and secondary metabolite biosynthesis (Wuyun et al., 2017).

2.2 Economic and medicinal importance

Eucommia ulmoides holds significant economic and medicinal value. It has the dual function of regulating blood pressure and other benefits, making it a traditional valuable traditional Chinese medicine. It is also valued for its ability to produce trans-1,4-polyisoprene rubber, also known as Eu-Rubber, which is synthesized in the tissues of leaves, bark, and fruit (Jin et al., 2020). This rubber, known as Eu-Rubber, is a high-molecular mass polymer of isoprene units with a trans-configuration, making it a valuable resource for various industrial applications (Wang et al., 2017). Additionally, the plant has been traditionally used in Chinese medicine for its purported health benefits, including anti-inflammatory, anti-fatigue, and anti-oxidative properties. The presence of secondary metabolites, which are crucial for its medicinal properties, is attributed to the extensive gene families involved in their biosynthesis (Wuyun et al., 2017).

2.3 Rubber production in *Eucommia ulmoides*

The rubber production in *Eucommia ulmoides* is a complex process involving several key enzymes and pathways. The synthesis of trans-polyisoprene rubber is hypothesized to be dominated by the mevalonate pathway, with farnesyl diphosphate synthase 2 (FPPS2) playing a crucial role in the biosynthesis of trans-polyprenyl diphosphate (Jin et al., 2020). The rubber elongation factor 3 (REF3) is also believed to be involved in stabilizing the membrane of rubber particles, which is essential for efficient rubber production (Jin et al., 2020). Recent studies have identified and characterized five novel FPS genes (EuFPS1-5) in *Eucommia ulmoides*, with EuFPS5 showing a strong correlation with the accumulation rate of Eu-Rubber, suggesting its pivotal role in rubber biosynthesis (Wang et al., 2017). This gene is identical to the EuFPS1 gene series that we cloned and named earlier (Zhou et al. 2003), and the EuFPS1 gene is regulated by the transcription factor EuTAG (Shi et al. 2024). Its interacting proteins include SRPP, REF, and various stress response proteins in response to environmental stimuli (Zhao et al., unpublished data). These findings provide a deeper understanding of the molecular mechanisms underlying rubber production in *Eucommia ulmoides* and highlight potential targets for genetic improvement and breeding programs aimed at enhancing rubber yield and quality.

3 Rubber Biosynthesis: General Mechanisms

3.1 Definition and types of natural rubber

Natural rubber is a polymer primarily composed of isoprene units. It exists in two main forms: cis-1,4-polyisoprene and trans-1,4-polyisoprene. The former is predominantly produced by the rubber tree, *Hevea brasiliensis*, and is widely used in commercial applications due to its elasticity and resilience. In contrast, *Eucommia ulmoides* (hardy rubber tree) produces trans-1,4-polyisoprene, which has unique properties that combine the characteristics of both rubber and plastic, making it a novel material with potential applications in various fields such as biomedical engineering and environmental sustainability (Wei et al., 2021).

3.2 Basic biochemical pathways of rubber biosynthesis

3.2.1 Isoprenoid Pathway

Rubber biosynthesis in *Eucommia ulmoides* involves the isoprenoid pathway, which can proceed via two distinct routes: the mevalonate (MVA) pathway and the methylerythritol phosphate (MEP) pathway. The MVA pathway operates in the cytosol, while the MEP pathway functions in the plastids. In *Eucommia ulmoides*, the MEP pathway is predominantly active in the leaves and central peels, contributing significantly to the synthesis of isoprenyl diphosphate, a precursor for trans-polyisoprene (Tokumoto et al., 2017; Li et al., 2020).

The study by Wuyun et. al. (2017), demonstrates the basic biochemical pathways involved in isoprenoid biosynthesis during rubber production. These pathways are divided into two main routes: the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Figure 1). The research indicates that rubber synthesis in *Eucommia ulmoides* (hardy rubber tree) primarily relies on the MVA pathway. Figure 1 clearly outlines the specific steps and key enzymes involved in both the MVA and MEP pathways, highlighting the central role of the MVA pathway in rubber biosynthesis. These findings not only deepen our understanding of the mechanisms of rubber biosynthesis but also provide important references for future genetic engineering and rubber production.

3.2.2 Polyisoprene formation

The formation of polyisoprene in *Eucommia ulmoides* involves the polymerization of isoprene units. This process is facilitated by enzymes such as trans-isoprenyl diphosphate synthase (TIDS), which catalyzes the formation of trans-1,4-polyisoprene. The biosynthesis of trans-polyisoprene is localized in specific tissues, such as the cambium layer, where it accumulates in fibrous structures (Bamba et al., 2002). The polymerization process is tightly regulated by various genes and enzymes, ensuring the efficient production of trans-polyisoprene (Chen et al., 2012; Wang et al., 2017).

3.3 Key enzymes and genes involved

Several key enzymes and genes are involved in the biosynthesis of rubber in *Eucommia ulmoides*. Farnesyl diphosphate synthase (FPS) is a crucial enzyme that catalyzes the production of farnesyl diphosphate, a key intermediate in the biosynthesis of terpenoids, including rubber. *Eucommia ulmoides* has multiple FPS genes (EuFPS1-5), with EuFPS5 being highly expressed in young fruit and correlating with the accumulation of trans-polyisoprene (Wang et al., 2017). Additionally, isopentenyl diphosphate isomerase (IPI) plays a vital role in converting isopentenyl diphosphate to its isomer, dimethylallyl diphosphate, which is essential for the initial steps of isoprenoid biosynthesis (Chen et al., 2012).

The coexpression network of genes involved in trans-polyisoprene biosynthesis includes both MVA and MEP pathway genes, indicating a coordinated regulation of these pathways. This network also involves long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), which play regulatory roles in gene expression and rubber biosynthesis (Tokumoto et al., 2017; Liu et al., 2018; Ye et al., 2019).

In summary, the biosynthesis of rubber in *Eucommia ulmoides* is a complex process involving multiple biochemical pathways and regulatory networks. Understanding these mechanisms provides valuable insights into the evolution and potential applications of natural rubber from this unique plant species.

4 Evolutionary Aspects of Rubber Biosynthesis in Plants

4.1 Comparative analysis with *Hevea brasiliensis* (Para Rubber Tree)

Eucommia ulmoides, commonly known as the hardy rubber tree, and Hevea brasiliensis, the Para rubber tree, are two significant sources of natural rubber, yet they produce different types of polyisoprene. E. ulmoides synthesizes trans-1,4-polyisoprene, whereas *H. brasiliensis* produces cis-1,4-polyisoprene. This difference in the type of polyisoprene is attributed to the distinct biosynthetic pathways and enzymes involved in each species. In *Eucommia ulmoides*, the farnesyl diphosphate synthases (FPSs) and rubber elongation factor/small rubber particle protein gene families have expanded independently from those in H. brasiliensis, indicating a divergent evolutionary pathway for rubber biosynthesis (Wuyun et al., 2017; Wei et al., 2021). Additionally, *Eucommia* *ulmoides* relies predominantly on the methylerythritol-phosphate (MEP) pathway for isoprenyl diphosphate synthesis, whereas H. brasiliensis primarily uses the mevalonate (MVA) pathway (Tokumoto et al., 2017; Li et al., 2020).

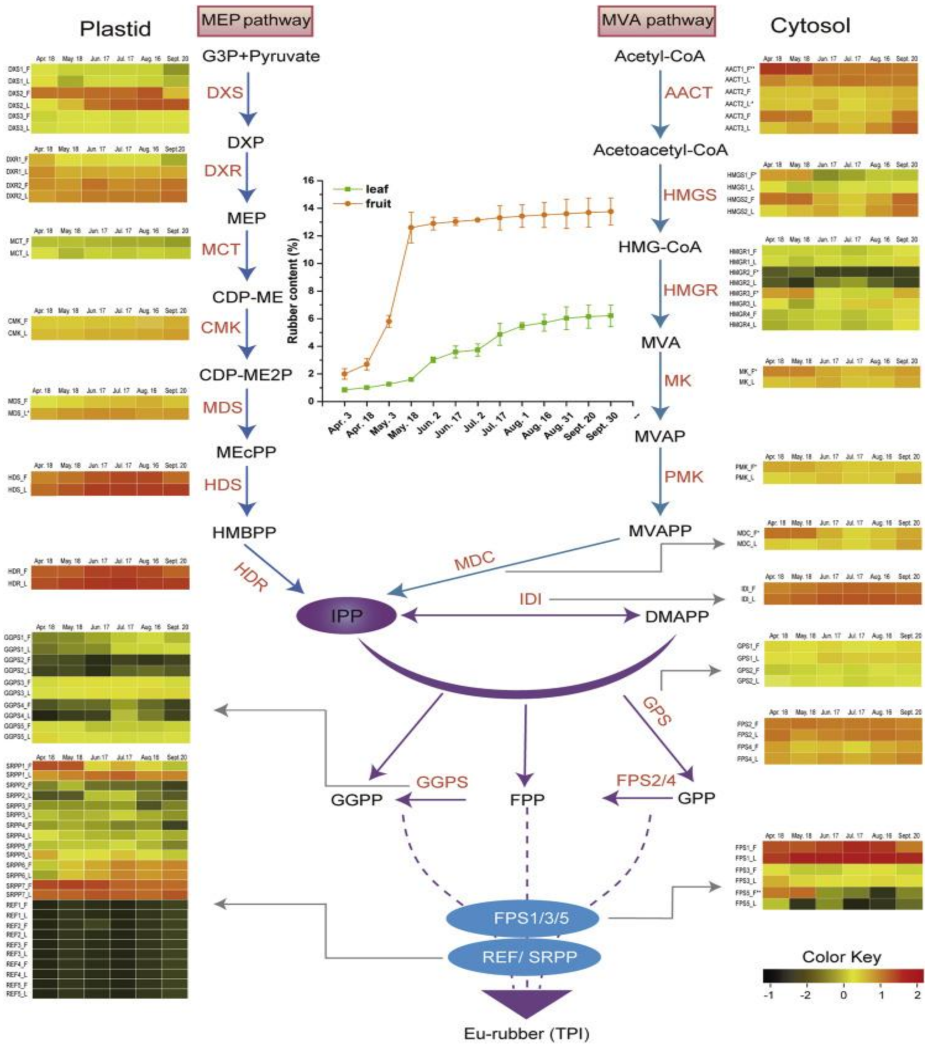

Figure 1 The Eu-rubber biosynthesis pathway and expression profiles of genes involved in the pathway (Adopted from Wuyun et. al., 2017)

Imagine Caption: Expression level is presented by log2-transformed fragments mapped per kilobase of transcript length per million total mapped reads (log2-FPKM). Asterisks indicate that the gene expression profile was correlated with the Eu-rubber accumulation at the *p < 0.05 and **p < 0.01 levels. Minus sign indicates that the gene expression profile was negatively correlated with the rubber accumulation. The curves in the center area show the Eu-rubber contents in fruits and leaves between April and September. L, leaf; F, fruit. AACT, acetyl-coenzyme A (CoA) C-acetyltransferase; HMGS, hydroxymethylglutaryl-CoA synthase; HMGR, hydroxymethylglutaryl-CoA reductase; MK, mevalonate kinase; PMK, 5-phosphomevalonate kinase; MDC, mevalonate pyrophosphate decarboxylase; DXS, 1-deoxy-d-xylulose 5-phosphate synthase; DXR, 1-deoxy-d-xylulose 5-phosphate reductoisomerase; MCT, 2-C-methyl-d-erythritol 4-phosphate cytidylyltransferase; CMK, 4-(cytidine 5′-diphospho)-2-C-methyl-d-erythritol kinase; MDS, 2-C-methyl-d-erythritol 2,4-cyclodiphosphate synthase; HDS, 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HDR, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; IDI, isopentenyl diphosphate isomerase; GPS, geranyl diphosphate synthase; FPS, farnesyl diphosphate synthase; GGPS, geranylgeranyl diphosphate synthase; REF, rubber elongation factor; SRPP, small rubber particle protein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MVA, mevalonate; MVAP, mevalonate-5-phosphate; MVAPP, mevalonate-5-diphosphate; G3P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-d-xylulose 5-phosphate; MEP, 2-C-methyl-d-erythritol 4-phosphate; CDP-ME, 4-(cytidine 5′-diphospho)-2-C-methyl-d-erythritol; CDP-ME2P, 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-d-erythritol; MEcPP, 2-C-methyl-d-erythritol 2,4-cyclodiphosphate; HMBPP, 4-hydroxy-3-methylbut-2-enyl diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate (Adopted from Wuyun et. al., 2017)

4.2 Phylogenetic relationships among rubber-producing plants

The phylogenetic analysis of rubber-producing plants reveals that *Eucommia ulmoides* is a sister taxon to lamiids and campanulids, and it underwent an ancient genome triplication shared by core eudicots approximately 125 million years ago. This triplication event is a significant evolutionary milestone that differentiates *Eucommia ulmoides* from other rubber-producing species like H. brasiliensis, which did not experience such an event (Wuyun et al., 2017). The unique evolutionary history of *Eucommia ulmoides*, including its genome triplication and subsequent lack of whole-genome duplication, has contributed to its distinct rubber biosynthesis pathway and environmental adaptability (Wuyun et al., 2017; Li et al., 2020).

4.3 Evolution of rubber biosynthesis pathways

The evolution of rubber biosynthesis pathways in *Eucommia ulmoides* involves the integration of both the MEP and MVA pathways, which is distinct from the pathway predominantly used by *H. brasiliensis*. In *Eucommia ulmoides*, the MEP pathway operates mainly in trans-polyisoprene-containing leaves and central peels, while the MVA pathway is also involved, indicating a more complex and integrated biosynthetic mechanism (Tokumoto et al., 2017; Li et al., 2020). The gene coexpression network analysis has shown that the TPI biosynthesis genes in *Eucommia ulmoides* are coordinately expressed and clustered into two modules, corresponding to the MEP and MVA pathways, respectively. This dual pathway system suggests an evolutionary adaptation that allows *Eucommia ulmoides* to efficiently produce trans-1,4-polyisoprene under various environmental conditions (Tokumoto et al., 2017).

The expansion of specific gene families, such as FPS and rubber elongation factor/small rubber particle protein genes, further highlights the evolutionary divergence of *Eucommia ulmoides* from other rubber-producing plants. These genetic adaptations have enabled *Eucommia ulmoides* to develop a unique rubber biosynthesis pathway that is distinct from the cis-polyisoprene production in *H. brasiliensis* (Wuyun et al., 2017; Li et al., 2020).

5 Molecular and Genetic Basis of Rubber Biosynthesis in *Eucommia ulmoides*

5.1 Key genes involved in rubber biosynthesis

5.1.1 Gene identification and annotation

The identification and annotation of key genes involved in rubber biosynthesis in *Eucommia ulmoides* have been significantly advanced by recent genomic studies. A high-quality assembly of the *Eucommia ulmoides* genome has revealed at least 26 723 predicted genes, providing a comprehensive resource for identifying genes involved in rubber biosynthesis (Wuyun et al., 2017). Additionally, a high-quality haploid chromosome-scale genome assembly has further refined the gene annotations, identifying 26 001 protein-coding genes anchored to 17 chromosomes (Li et al., 2020). These genomic resources have facilitated the identification of key genes such as farnesyl diphosphate synthases (FPSs) and rubber elongation factor/small rubber particle protein (REF/SRPP) gene families, which are crucial for rubber biosynthesis (Wang et al., 2017; Wuyun et al., 2017).

5.1.2 Functional characterization of key genes

Functional characterization of these genes has revealed their specific roles in rubber biosynthesis. For instance, five novel FPS genes (EuFPS1-5) have been cloned and characterized, showing differential expression patterns across various tissues. EuFPS5, in particular, is highly expressed in young fruit and correlates with the accumulation rate of *Eucommia* rubber (Eu-Rubber), suggesting its pivotal role in rubber biosynthesis (Wang et al., 2017). Additionally, the expression of small rubber particle protein 1 (EuSRPP1) has been linked to higher rubber accumulation, regulated by microRNAs such as Eu-miR45 (Liu et al., 2022).

5.2 Gene expression profiles in rubber-producing tissues

Gene expression profiling in rubber-producing tissues has provided insights into the spatial and temporal regulation of rubber biosynthesis. High-throughput RNA sequencing has identified differentially expressed genes (DEGs) in tissues such as leaves, bark, and fruit, which are involved in terpenoid biosynthesis pathways (Jin et al., 2020). For example, the methylerythritol-phosphate (MEP) pathway operates predominantly in trans-polyisoprene-containing leaves and central peels, while the mevalonate (MVA) pathway is also implicated in rubber biosynthesis (Tokumoto et al., 2017; Li et al., 2020). These findings highlight the complex regulation of rubber biosynthesis at the transcriptional level.

5.3 Genomic and transcriptomic studies

5.3.1 Whole Genome Sequencing

Whole genome sequencing of *Eucommia ulmoides* has provided a foundational understanding of its genetic makeup and evolutionary history. The initial genome assembly revealed a ~ 1.2-Gb genome with high expression levels of genes involved in stress responses and secondary metabolite biosynthesis (Wuyun et al., 2017). A subsequent high-quality haploid genome assembly improved the sequence contiguity and identified a new whole-genome duplication event, contributing to the evolution of the genome and the expansion of long terminal repeats (Li et al., 2020).

5.3.2 RNA-seq analysis

RNA-Seq analysis has been instrumental in uncovering the gene expression dynamics associated with rubber biosynthesis. Transcriptome sequencing of various tissues has identified numerous unigenes involved in terpenoid biosynthesis, with specific genes such as farnesyl diphosphate synthase 2 (FPPS2) and rubber elongation factor 3 (REF3) playing key roles (Jin et al., 2020). Additionally, the coexpression network analysis has revealed that genes involved in the MEP and MVA pathways are coordinately expressed, indicating their collaborative role in trans-polyisoprene biosynthesis (Tokumoto et al., 2017).

In summary, the molecular and genetic basis of rubber biosynthesis in *Eucommia ulmoides* is underpinned by a complex interplay of key genes, their functional characterization, and their expression profiles in rubber-producing tissues. Genomic and transcriptomic studies have provided a comprehensive framework for understanding and potentially manipulating this economically important biosynthetic pathway.

6 Biochemical Pathways Specific to *Eucommia ulmoides*

6.1 Unique aspects of *Eucommia ulmoides* rubber biosynthesis

Eucommia ulmoides synthesizes trans-polyisoprene rubber, which is distinct from the cis-polyisoprene produced by Hevea brasiliensis. This unique biosynthesis pathway in *Eucommia ulmoides* primarily utilizes the methylerythritol-phosphate (MEP) pathway rather than the mevalonate pathway to synthesize isoprenyl diphosphate, a key precursor in rubber biosynthesis (Wuyun et al., 2017; Li et al., 2020). The MEP pathway operates predominantly in the leaves and central peels of *Eucommia ulmoides*, highlighting a significant divergence from other rubber-producing species (Li et al., 2020). Additionally, the farnesyl diphosphate synthase (FPS) gene family in *Eucommia ulmoides* has undergone independent expansion, which is crucial for the synthesis of long-chain trans-polyisoprene (Wuyun et al., 2017).

6.2 Enzyme activity and metabolic flux analysis

The enzyme farnesyl diphosphate synthase (FPS) plays a pivotal role in the biosynthesis of trans-polyisoprene in *Eucommia ulmoides*. Five novel FPS genes (EuFPS1-5) have been identified, each exhibiting distinct expression patterns across different tissues and developmental stages. Notably, EuFPS5 is highly expressed in young fruit and correlates with the accumulation rate of Eu-rubber, suggesting its critical role in rubber biosynthesis (Wang et al., 2017). Furthermore, the rubber elongation factor (REF) and small rubber particle protein (SRPP) gene families have also expanded in *Eucommia ulmoides*, contributing to the stabilization and elongation of rubber particles (Wuyun et al., 2017; Jin et al., 2020). Metabolic flux analysis indicates that the MEP pathway enzymes are preferentially expressed in leaves, which aligns with the high rubber content observed in these tissues (Li et al., 2020).

6.3 Secondary metabolites and their roles

Secondary metabolites play essential roles in the overall biosynthesis and regulation of rubber in *Eucommia ulmoides*. Chlorogenic acid biosynthesis pathway enzymes, for instance, are preferentially expressed in the leaves, suggesting a potential role in protecting the rubber biosynthesis machinery from oxidative stress (Li et al., 2020). Additionally, long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) have been identified as key regulators in the rubber biosynthesis process. These non-coding RNAs form complex regulatory networks that modulate the expression of genes involved in rubber production, such as the small rubber particle protein 1 (EuSRPP1) (Liu et al., 2018; Ye et al., 2019; Liu et al., 2022). The interplay between these secondary metabolites and regulatory RNAs underscores the sophisticated control mechanisms governing rubber biosynthesis in *Eucommia ulmoides*.

7 Environmental and Physiological Influences on Rubber Biosynthesis

7.1 Impact of environmental factors

7.1.1 Temperature

Temperature plays a crucial role in the biosynthesis of rubber in *Eucommia ulmoides*. The metabolic pathways involved in rubber production, such as the methylerythritol-phosphate (MEP) pathway, are temperature-sensitive. High temperatures can enhance the activity of enzymes involved in these pathways, thereby increasing rubber yield. Conversely, low temperatures may inhibit enzyme activity, reducing rubber production (Tokumoto et al., 2017; Li et al., 2020).

7.1.2 Light

Light is another significant environmental factor influencing rubber biosynthesis. The presence of light-responsive elements in the promoter regions of key biosynthetic genes suggests that light can regulate the expression of these genes. This regulation is crucial for the synthesis of trans-polyisoprene, as light can affect the photosynthetic activity and energy availability required for the biosynthetic processes (Liu et al., 2018; Hu et al., 2023).

7.1.3 Soil nutrients

Soil nutrients, particularly nitrogen, phosphorus, and potassium, are essential for the optimal growth and rubber production in *Eucommia ulmoides*. These nutrients support various physiological processes, including the synthesis of isoprenoid precursors. Adequate nutrient supply can enhance the expression of genes involved in the MEP and mevalonate (MVA) pathways, thereby boosting rubber biosynthesis (Wang et al., 2017; Jin et al., 2020).

7.2 Physiological conditions and stress responses

7.2.1 Water stress

Water stress significantly impacts rubber biosynthesis in *Eucommia ulmoides*. Drought conditions can lead to the accumulation of abscisic acid (ABA), which in turn affects the expression of genes involved in rubber biosynthesis. The presence of ABA-responsive elements in the promoter regions of these genes indicates that water stress can modulate their expression, thereby influencing rubber production (Liu et al., 2018; Hu et al., 2023).

7.2.2 Pathogen attack

Pathogen attacks can trigger complex stress responses in *Eucommia ulmoides*, affecting rubber biosynthesis. The plant's defense mechanisms involve the upregulation of stress-responsive genes, some of which are also involved in rubber biosynthesis. This dual role suggests that pathogen-induced stress can either enhance or inhibit rubber production, depending on the specific stress response pathways activated (Wuyun et al., 2017; Wei et al., 2021).

7.3 Adaptation mechanisms in *Eucommia ulmoides*

Eucommia ulmoides has evolved several adaptation mechanisms to cope with environmental and physiological stresses, thereby ensuring consistent rubber production. The expansion of gene families involved in stress responses and secondary metabolite biosynthesis is one such mechanism. These genetic adaptations enable the plant to maintain high levels of rubber biosynthesis even under adverse conditions (Wang et al., 2017; Wuyun et al., 2017; Li et al., 2020). Additionally, the co-expression of genes involved in both the MEP and MVA pathways provides a robust metabolic network that can adapt to varying environmental conditions, ensuring the continuous production of trans-polyisoprene (Tokumoto et al., 2017; Nakazawa et al., 2009).

These insights into the environmental and physiological influences on rubber biosynthesis in *Eucommia ulmoides* highlight the plant's remarkable adaptability and the complex regulatory networks that sustain its rubber production capabilities.

8 Applications and Biotechnological Advances

8.1 Biotechnological enhancement of rubber production

Eucommia ulmoides has garnered significant attention due to its ability to produce trans-polyisoprene rubber, a valuable biopolymer. Recent advancements in biotechnological methods have paved the way for enhancing rubber production in this species. The high-quality genome assembly of *Eucommia ulmoides* has provided a comprehensive understanding of the genetic basis of rubber biosynthesis, enabling targeted genetic improvements (Wuyun et al., 2017; Li et al., 2020). Transcriptome analysis has identified key genes involved in the terpenoid biosynthesis pathway, such as farnesyl diphosphate synthase (FPS) and rubber elongation factor (REF), which are crucial for rubber production (Jin et al., 2020). These insights facilitate the development of molecular markers and genetic maps, which can be used to breed *Eucommia ulmoides* varieties with enhanced rubber yield and quality (Jin et al., 2020).

8.2 Genetic engineering approaches

Genetic engineering offers promising avenues for improving rubber biosynthesis in *Eucommia ulmoides*. The identification and characterization of the FPS gene family have revealed that specific isoforms, such as EuFPS5, are highly expressed in young fruit and correlate with rubber accumulation (Wang et al., 2017). By manipulating the expression of these key genes, it is possible to enhance the biosynthetic pathway and increase rubber production. Additionally, the high-quality haploid genome assembly has highlighted the role of the methylerythritol-phosphate (MEP) pathway in rubber biosynthesis, providing new targets for genetic modification (Li et al., 2020). Advanced techniques such as CRISPR/Cas9 can be employed to edit these genes, potentially leading to *Eucommia ulmoides* varieties with superior rubber-producing capabilities.

8.3 Potential industrial applications

The unique properties of *Eucommia ulmoides* rubber (EU-rubber) make it a valuable material for various industrial applications. EU-rubber is a hard rubber with thermoplastic properties, making it suitable for use as an industrial raw material to substitute for petroleum-based products (Nakazawa et al., 2009). Recent studies have demonstrated the potential of modified EU-rubber in creating sustainable elastomers with enhanced elasticity and shape memory capabilities, which could be used in tires and smart materials (Zhang et al., 2019). Furthermore, the dual rubber-plastic nature of EU-rubber opens up possibilities for its application in biomedicine, textiles, and aerospace (Wang et al., 2020). The ability to regulate the crystallinity and cross-linking degree of EU-rubber through physical or chemical modifications allows for the development of engineering materials that meet specific performance requirements, further expanding its industrial applications (Wang et al., 2020).

9 Future Directions and Research Prospects

9.1 Unresolved questions in rubber biosynthesis

Despite significant advancements in understanding the biosynthesis of rubber in *Eucommia ulmoides*, several questions remain unresolved. One critical area of inquiry is the precise regulatory mechanisms governing the expression of key genes involved in rubber biosynthesis. For instance, while the role of farnesyl diphosphate synthase (FPS) and rubber elongation factor (REF) genes has been established, the upstream regulatory networks and environmental factors influencing their expression are not fully understood (Wuyun et al., 2017; Jin et al., 2020). Additionally, the involvement of long non-coding RNAs (lncRNAs) in the regulation of rubber biosynthesis presents another layer of complexity. Recent studies have identified numerous lncRNAs and transcripts of uncertain coding potential (TUCPs) that may play pivotal roles in this process, but their exact functions and interactions with protein-coding genes require further elucidation (Liu et al., 2018).

9.2 Emerging technologies and methodologies

The advent of high-throughput sequencing technologies and advanced bioinformatics tools has opened new avenues for exploring the genetic and molecular basis of rubber biosynthesis in *Eucommia ulmoides*. The integration of Illumina sequencing, PacBio sequencing, and BioNano mapping has already facilitated the assembly of a high-quality genome for *Eucommia ulmoides*, providing a valuable resource for future research (Wuyun et al., 2017). Transcriptome analysis using platforms like the Illumina HiSeq 2000 system has enabled the identification of differentially expressed genes and unigenes involved in terpenoid biosynthesis, offering insights into the metabolic pathways leading to rubber production (Jin et al., 2020). Moreover, the application of RNA deep-sequencing has revealed the potential regulatory roles of lncRNAs and TUCPs, suggesting new targets for genetic manipulation (Liu et al., 2018). These emerging technologies will be instrumental in dissecting the complex biosynthetic pathways and regulatory networks in *Eucommia ulmoides*.

9.3 Integrative approaches for comprehensive understanding

To achieve a comprehensive understanding of rubber biosynthesis in *Eucommia ulmoides*, integrative approaches combining genomics, transcriptomics, proteomics, and metabolomics are essential. By leveraging the high-quality genome assembly and extensive transcriptome data, researchers can perform comparative analyses to identify conserved and unique elements of rubber biosynthesis pathways across different species (Wuyun et al., 2017; Jin et al., 2020). Functional genomics studies, including gene knockout and overexpression experiments, will help elucidate the roles of specific genes and regulatory elements. Additionally, integrating proteomic and metabolomic data will provide a holistic view of the biochemical processes and metabolic fluxes involved in rubber production. Such multidisciplinary approaches will not only enhance our understanding of the fundamental biology of *Eucommia ulmoides* but also pave the way for biotechnological applications aimed at improving rubber yield and quality.

In conclusion, while significant progress has been made in unraveling the genetic and molecular basis of rubber biosynthesis in *Eucommia ulmoides*, many questions remain unanswered. The continued development and application of advanced technologies, coupled with integrative research approaches, will be crucial in addressing these gaps and unlocking the full potential of this economically important species.

10 Concluding Remarks

The research on *Eucommia ulmoides* has provided significant insights into the evolutionary pathways of rubber biosynthesis. The high-quality genome assembly of *Eucommia ulmoides* revealed that it has undergone an ancient genome triplication but no further whole-genome duplication in the last 125 million years. This genomic stability has allowed for the expansion and high expression of genes involved in stress responses and secondary metabolite biosynthesis, which contribute to its environmental adaptability. The biosynthesis of trans-polyisoprene rubber in *Eucommia ulmoides* is primarily driven by the mevalonate pathway, with farnesyl diphosphate synthase (FPS) playing a crucial role. The identification and characterization of five novel FPS genes (EuFPS1-5) have highlighted their differential expression across various tissues, with EuFPS5 being particularly significant in young fruit, correlating with rubber accumulation. Additionally, long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) have been identified as key regulatory elements in the rubber biosynthesis process, providing a complex regulatory network that controls gene expression.

The findings from these studies have several implications for rubber production. The detailed genomic and transcriptomic data can be leveraged to enhance the genetic engineering of *Eucommia ulmoides*, potentially increasing the yield and quality of trans-polyisoprene rubber. The identification of key genes and regulatory networks involved in rubber biosynthesis opens up new avenues for targeted genetic modifications. For instance, manipulating the expression of FPS genes, particularly EuFPS5, could lead to higher rubber content in specific tissues. Moreover, the understanding of lncRNAs and miRNAs in regulating rubber biosynthesis provides additional targets for genetic interventions. The development of modified *Eucommia ulmoides* gum (EUG) with enhanced mechanical properties and shape memory capabilities further extends the potential applications of this natural rubber in various industries, including tires and smart materials.

The research on *Eucommia ulmoides* has significantly advanced our understanding of the evolutionary pathways and molecular mechanisms underlying rubber biosynthesis. The integration of genomic, transcriptomic, and regulatory data provides a comprehensive framework for future studies aimed at improving rubber production. Future research should focus on functional validation of the identified genes and regulatory elements, as well as exploring the potential of CRISPR/Cas9 and other gene-editing technologies to enhance rubber yield and quality. Additionally, the development of sustainable and high-performance rubber products from E. ulmoides gum holds promise for expanding its industrial applications. Continued interdisciplinary efforts will be crucial in translating these scientific insights into practical advancements in rubber production and utilization.

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Conflict of Interest Disclosure

The author affirms 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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