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Comparative Study of Rubber Biosynthesis Pathways in *Eucommia ulmoides* and *Hevea brasiliensis*

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Abstract This study conducts a comparative analysis of the rubber biosynthesis pathways in two significant rubber-producing species, *Eucommia ulmoides* and *Hevea brasiliensis*. By examining the genetic and biochemical mechanisms underlying rubber production in these species, the study aims to uncover the evolutionary adaptations and potential biotechnological applications of their distinct biosynthetic pathways. The study reveals that *Eucommia ulmoides* primarily utilizes the methylerythritol-phosphate (MEP) pathway for isoprenyl diphosphate synthesis, which is a precursor for trans-polyisoprene rubber. In contrast, *Hevea brasiliensis* predominantly employs the mevalonate (MVA) pathway for cis-polyisoprene rubber production. Additionally, the farnesyl diphosphate synthase (FPS) gene families in both species show significant differences in expression patterns and gene expansions, which are crucial for their respective rubber biosynthesis processes. The study also identifies long non-coding RNAs (lncRNAs) and microRNAs that play regulatory roles in rubber biosynthesis, providing deeper insights into the molecular regulation of this process. The findings highlight the evolutionary divergence in rubber biosynthesis pathways between *Eucommia ulmoides* and *Hevea brasiliensis*. Understanding these differences not only enriches our knowledge of plant secondary metabolism but also opens up new avenues for genetic engineering to enhance rubber production in these and other species. The study underscores the potential for biotechnological advancements in the rubber industry by leveraging the unique biosynthetic pathways of these plants.

Keywords Rubber biosynthesis; *Eucommia ulmoides*; *Hevea brasiliensis*; MEP pathway; MVA pathway; Farnesyl diphosphate synthase; Long non-coding RNAs; microRNAs; Genetic engineering

1 Introduction

Natural rubber is a critical raw material with extensive applications in various industries, including automotive, medical, and defense. The primary source of natural rubber is the Para rubber tree, *Hevea brasiliensis*, which produces cis-1,4-polyisoprene. This biopolymer is valued for its high elasticity, flexibility, and resilience, making it indispensable for manufacturing over 50 000 rubber products, such as tires and medical gloves (Rahman et al., 2013; Cherian et al., 2019). The global demand for natural rubber continues to rise, driven by its unique properties that synthetic alternatives cannot fully replicate (Cherian et al., 2019). Consequently, understanding and improving rubber biosynthesis pathways is of paramount economic importance.

Hevea brasiliensis is the most widely cultivated species for commercial natural rubber production. The biosynthesis of rubber in *H. brasiliensis* occurs through the mevalonate (MVA) pathway, which provides isopentenyl diphosphate (IPP) for cis-polyisoprene synthesis (Chow et al., 2007; Chow et al., 2011; Ambily et al., 2019). The genome of *H. brasiliensis* has been extensively studied, revealing a significant expansion of the rubber elongation factor (REF) and small rubber particle protein (SRPP) gene families, which are crucial for rubber biosynthesis (Lau et al., 2016; Tang et al., 2016).

In contrast, *Eucommia ulmoides*, known for its medicinal applications, produces trans-1,4-polyisoprene, an isomer of natural rubber. The rubber biosynthesis in *E. ulmoides* primarily relies on the methylerythritol-phosphate (MEP) pathway rather than the MVA pathway. Recent genomic studies have provided high-quality assemblies of the *E.*

ulmoides genome, offering new insights into its rubber biosynthesis mechanisms and evolutionary history (Li et al., 2020). The unique properties of *E. ulmoides* rubber (EUR) have garnered increasing attention for its potential applications in various fields, including environment, agriculture, engineering, and biomedical engineering (Wei et al., 2021).

Eucommia ulmoides is a typical dioecious tree species whose metabolomics and transcriptomics have been well analyzed (Li et al., 2024). Natural rubber consists of polyisoprene, including trans-polyisoprene (TPI) and cis-polyisoprene (CPI). Eucommia rubber, found in the leaves, bark, fruit peel, and roots of Eucommia, is a high-quality natural rubber resource that accumulates trans-polyisoprene (Ma et al., 2024). It possesses excellent abrasion resistance and aging resistance (Wang et al., 2021) and serves as an efficient reinforcing agent (Liu et al., 2022). Eucommia rubber can be developed into functional materials with electromagnetic shielding or shape-memory properties (Qi et al., 2023).

This study explores the differences and similarities in the biosynthetic mechanisms of *Eucommia ulmoides* to reveal the genetic and biochemical factors influencing various rubber production processes. The research focuses on the roles of the MVA and MEP pathways in IPP synthesis and the expression of key genes involved in rubber biosynthesis. This study will provide valuable insights into the molecular basis of rubber biosynthesis in *Eucommia ulmoides* and *Hevea brasiliensis*. Understanding these pathways may offer a theoretical foundation for developing genetically engineered plants with improved rubber production, thereby enhancing rubber yield and quality. Additionally, the findings may provide new perspectives on the evolutionary adaptations of these species and their potential applications in various industrial and medical fields.

2 Genomic Insights into Rubber Biosynthesis

2.1 *Eucommia ulmoides* genome

The *Eucommia ulmoides* genome has been successfully assembled to a high-quality haploid chromosome-scale, marking a significant milestone in genomic research for tree species. This assembly was achieved using PacBio and Hi-C technologies, resulting in a scaffold N50 of 53.15 MB, a 28-fold increase from previous assemblies. The repetitive sequence content also saw a substantial increase, and the number of gaps decreased dramatically, enhancing the overall quality and contiguity of the genome sequence. This high-quality assembly is pivotal for advancing studies on genome structure, evolution, gene mapping, and functional genomics, and it holds promise for improving *E. ulmoides* for industrial and medical applications through genetic engineering (Li et al., 2020).

The genome of *E. ulmoides* has provided valuable insights into its rubber biosynthesis pathways. Unlike *Hevea brasiliensis*, which relies on the mevalonate pathway, *E. ulmoides* predominantly uses the methylerythritol-phosphate (MEP) pathway to synthesize isoprenyl diphosphate. This pathway is mainly active in trans-polyisoprene-containing leaves and central peels. Additionally, the genome revealed that enzymes involved in chlorogenic acid biosynthesis are preferentially expressed in leaves rather than in bark, indicating tissue-specific metabolic activities (Wuyun et al., 2017; Li et al., 2020).

The *E. ulmoides* genome has undergone significant evolutionary events, including a new whole-genome duplication superimposed on an earlier γ paleohexaploidization event. Furthermore, an ancient genome triplication shared by core eudicots was identified, but no further whole-genome duplications have occurred in the last approximately 125 million years. These duplication and triplication events have contributed to the expansion of gene families involved in stress responses and secondary metabolite biosynthesis, enhancing the environmental adaptability of *E. ulmoides* (Wuyun et al., 2017; Li et al., 2020).

2.2 *Hevea brasiliensis* Genome

The genome of *Hevea brasiliensis*, the primary commercial source of natural rubber, has been sequenced and assembled, covering approximately 1.1 Gb of the estimated 2.15 Gb haploid genome. This draft genome sequence has identified around 68,955 gene models, with 12.7% being unique to Hevea. The comprehensive genome analysis has provided crucial insights into the genetic basis of rubber biosynthesis, rubberwood formation, disease resistance, and allergenicity. This genomic information is essential for developing high-yielding clones to meet the growing demand for natural rubber (Rahman et al., 2013).

In *H. brasiliensis*, gene families associated with rubber biosynthesis have undergone significant expansion. Notably, genes encoding rubber particle membrane proteins (RPMPs), including rubber elongation factor (REF) and small rubber particle protein (SRPP), are highly expressed in latex. These proteins play critical roles in the biosynthesis of cis-polyisoprene, the primary component of natural rubber. The expansion and high expression levels of these gene families underscore their importance in rubber production and stress response mechanisms (Chow et al., 2007; Chuntai et al., 2017).

Comparative genomics between *H. brasiliensis* and other Euphorbiaceae species has revealed unique evolutionary adaptations in rubber biosynthesis. For instance, while *E. ulmoides* synthesizes trans-polyisoprene via farnesyl diphosphate synthases (FPSs), *H. brasiliensis* produces cis-polyisoprene. The independent expansion of FPS and rubber elongation factor gene families in *H. brasiliensis* highlights the divergent evolutionary paths taken by these species to optimize rubber production. These comparative studies provide a deeper understanding of the genetic and biochemical diversity within the Euphorbiaceae family (Wuyun et al., 2017; Chow et al., 2020).

3 Biochemical Pathways of Rubber Biosynthesis

3.1 Pathways in *Eucommia ulmoides*

In *Eucommia ulmoides*, the methylerythritol-phosphate (MEP) pathway is the primary route for the biosynthesis of isoprenoids, which are crucial for rubber production. This pathway operates in the plastids and is responsible for the synthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the fundamental building blocks for polyisoprene synthesis. The predominance of the MEP pathway in *E. ulmoides* highlights its essential role in the production of trans-polyisoprene rubber, a high-molecular mass polymer of isoprene units with a trans-configuration (Kajiura et al., 2017; Wang et al., 2017).

It is known that the biosynthesis pathway of trans-polyisoprene (TPI) in *Eucommia ulmoides* involves 47 genes, including genes from the MEP and MVA pathways, geranyl diphosphate synthase (GPS), geranylgeranyl diphosphate synthases (GGPSs), farnesyl pyrophosphate synthases (FPSs), and rubber elongation genes (Wuyun et al., 2018; Li et al., 2020).

Farnesyl diphosphate synthases (FPSs) are key enzymes in the biosynthesis of prenyl precursors, which are vital for the production of various terpenoids, including rubber. In *E. ulmoides*, multiple FPS genes have been identified and characterized, with distinct enzymatic properties and expression patterns. For instance, EuFPS1 and EuFPS2 exhibit different substrate preferences and reaction products, which influence the synthesis of farnesyl diphosphate (FPP) and its elongation to geranylgeranyl diphosphate. These differences in FPS activity are regulated by factors such as pH, metal ion cofactors, and cofactor concentrations, ultimately affecting the biosynthesis of trans-polyisoprene (Kajiura et al., 2017; Wang et al., 2017).

Eucommia rubber is a secondary metabolite of *Eucommia ulmoides*, but its transcriptional regulatory mechanisms for biosynthesis remain unclear. The biosynthesis of Eucommia rubber is regulated by multiple genes, such as EuFPS1 (farnesyl diphosphate synthase), a key enzyme in the biosynthetic process of Eucommia rubber. EuFPS1 is positively regulated by EuWRKY30, which plays a critical role in the synthesis of Eucommia rubber (Zhang et al., 2024).

3.2 Pathways in *Hevea brasiliensis*

In *Hevea brasiliensis*, the conventional mevalonate (MVA) pathway is the primary route for the synthesis of isoprenoids, including cis-polyisoprene, the main component of natural rubber. This pathway operates in the cytosol and involves the conversion of acetyl-CoA to IPP and DMAPP through a series of enzymatic reactions. The MVA pathway's role in cis-polyisoprene synthesis is crucial, as it provides the necessary precursors for the polymerization process (Chuntai et al., 2017).

Although the MVA pathway is the primary route for isoprenoid biosynthesis in *H. brasiliensis*, there is evidence suggesting a potential role for the MEP pathway in supplying IPP. This alternative pathway, which operates in the plastids, may contribute to the overall pool of IPP and DMAPP, thereby supporting the biosynthesis of

cis-polyisoprene. The interplay between the MVA and MEP pathways in *H. brasiliensis* highlights the complexity of isoprenoid biosynthesis and its regulation (Chuntai et al., 2017).

Subcellular compartmentalization plays a significant role in the biosynthesis of rubber in *H. brasiliensis*. The separation of the MVA pathway in the cytosol and the MEP pathway in the plastids allows for the distinct regulation and coordination of isoprenoid biosynthesis. This compartmentalization ensures the efficient production of IPP and DMAPP, which are essential for the synthesis of cis-polyisoprene. Additionally, the expression of FPS genes in different tissues and their regulation by environmental factors further influence rubber biosynthesis in *H. brasiliensis* (Chuntai et al., 2017).

By understanding the biochemical pathways and the role of key enzymes in rubber biosynthesis, researchers can develop strategies to enhance rubber production in both *Eucommia ulmoides* and *Hevea brasiliensis*.

4 Molecular Regulation of Rubber Biosynthesis

4.1 Gene expression and regulation in *Eucommia ulmoides*

Eucommia ulmoides exhibits high expression levels of multiple genes involved in stress responses and the biosynthesis of secondary metabolites, which may contribute to its significant environmental adaptability. This includes the expansion of gene families related to farnesyl diphosphate synthases (FPSs) and rubber elongation factors, which are crucial for the biosynthesis of trans-polyisoprene, the primary component of Eu-rubber (Wuyun et al., 2017) (Figure 1). Additionally, the NAC transcription factor family in *E. ulmoides*, which is involved in various stress responses and secondary metabolite synthesis, shows differential expression across various tissues, indicating their potential role in rubber biosynthesis (Zhang et al., 2023).

Several key transcription factors (TFs) involved in regulating the metabolism of Brazilian CPI have been identified, including WRKY, MADS, NAC, and MYB. These TFs regulate the expression of synthase genes related to natural rubber biosynthesis (Wang et al., 2013; Li et al., 2016; Cao et al., 2017; Wang et al., 2017). The expression of EuFPS1 in Eucommia is transcriptionally activated by EuWRKY30, and overexpression of EuWRKY30 significantly increases the expression level of EuFPS1, thereby promoting the biosynthesis of TPI (Zhang et al., 2024).

In *E. ulmoides*, enzymes involved in the chlorogenic acid biosynthesis pathway are preferentially expressed in the leaves rather than in the bark. This suggests a tissue-specific regulation of secondary metabolite biosynthesis, which may be linked to the plant's overall metabolic strategy and adaptation mechanisms (Li et al., 2020).

4.2 Gene expression and regulation in *Hevea brasiliensis*

In *Hevea brasiliensis*, long noncoding RNAs (lncRNAs) and microRNAs exhibit differential expression across various clones, indicating their regulatory roles in rubber biosynthesis. These noncoding RNAs are involved in complex regulatory networks that control the expression of genes associated with latex production and stress responses (Liu et al., 2018).

Hevea brasiliensis has undergone an expansion of genes related to rubber biosynthesis, particularly those encoding rubber particle membrane proteins (RPMPs) such as rubber elongation factor (REF) and small rubber particle protein (SRPP). These genes are highly expressed in latex, underscoring their critical role in the production of cis-polyisoprene, the main component of natural rubber (Chow et al., 2007). Additionally, farnesyl pyrophosphate synthase (FPS) genes in *H. brasiliensis* are highly expressed in latex and are upregulated in response to tapping and hormonal treatments, further highlighting their importance in rubber biosynthesis (Chuntai et al., 2017).

The transcription profiles of rubber biosynthesis-related genes in *H. brasiliensis* are highly tissue-specific. For instance, the TGA transcription factors, particularly HbTGA1, regulate the expression of multiple rubber biosynthesis genes in latex. These transcription factors bind to the promoters of key biosynthetic genes and modulate their activity in response to stress signals such as jasmonate and salicylic acid, indicating a sophisticated level of transcriptional regulation (Guo et al., 2022). This tissue-specific and alternative transcriptional regulation ensures the efficient production of natural rubber in the appropriate cellular context.

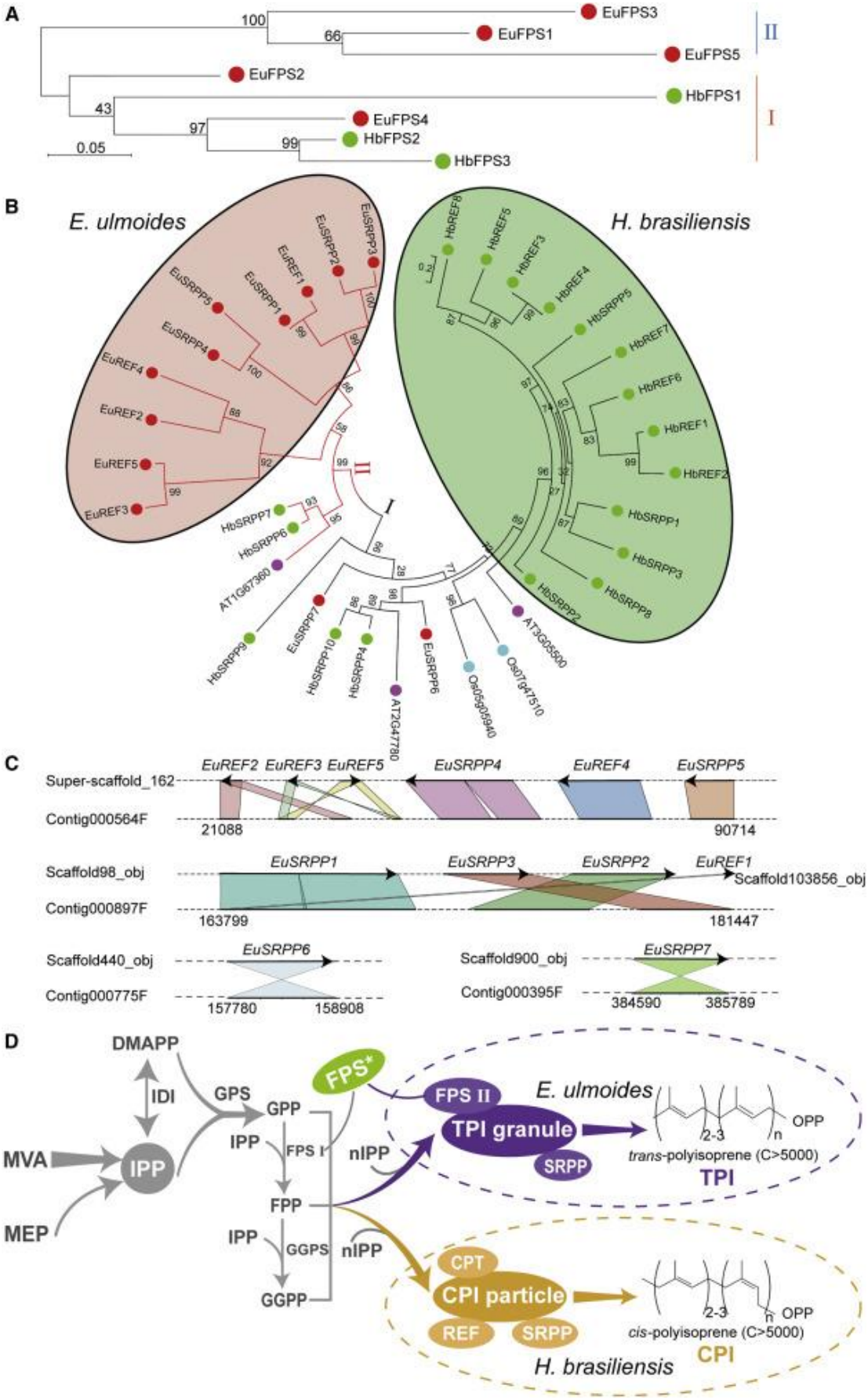

Figure 1 Phylogenetic Analyses, gene distribution, and comparison of rubber biosynthesis pathways (Adopted from Wuyun et al., 2017)

5 Comparative Analysis

5.1 Evolutionary divergence

Eucommia ulmoides and *Hevea brasiliensis* have distinct evolutionary paths that have influenced their rubber biosynthesis capabilities. *E. ulmoides*, a member of the order Garryales, has not undergone whole-genome duplication in the last 125 million years, unlike many other eudicots. This ancient genome triplication is shared among core eudicots but is unique in its lack of further duplications (Wuyun et al., 2017). In contrast, *H. brasiliensis*, which belongs to the order Malpighiales, has a more recent evolutionary history with significant genome duplications that have contributed to its current genetic makeup (Chow et al., 2007).

5.2 Biochemical pathway divergence

The biosynthesis of rubber in *E. ulmoides* and *H. brasiliensis* involves different primary pathways for the production of isopentenyl diphosphate (IPP), a key precursor. *E. ulmoides* predominantly utilizes the methylerythritol-phosphate (MEP) pathway for IPP synthesis, which is mainly active in the leaves and central peels (Li et al., 2020). This pathway is less common in rubber-producing plants and highlights a unique aspect of *E. ulmoides*' biochemistry. On the other hand, *H. brasiliensis* primarily relies on the mevalonate (MVA) pathway for IPP production in its latex, although the MEP pathway also contributes to a lesser extent, particularly in relation to carotenoid synthesis (Chow et al., 2011).

The farnesyl diphosphate synthases (FPSs) and rubber elongation factors (REFs) play crucial roles in the rubber biosynthesis of both species. In *E. ulmoides*, the FPS and rubber elongation factor/small rubber particle protein (SRPP) gene families have expanded independently from those in *H. brasiliensis*, leading to the production of trans-polyisoprene (Wuyun et al., 2017). This is in stark contrast to *H. brasiliensis*, where the FPSs and REFs are more closely associated with the synthesis of cis-polyisoprene. The latex of *H. brasiliensis* contains multiple isoforms of REFs and SRPPs, which are highly expressed and play significant roles in rubber particle formation and stability (Chow et al., 2007).

5.3 Regulatory mechanisms

The regulatory mechanisms governing rubber biosynthesis in *E. ulmoides* and *H. brasiliensis* also exhibit notable differences. In *E. ulmoides*, the high expression levels and gene number expansion for stress response and secondary metabolite biosynthesis genes suggest a complex regulatory network that enhances its environmental adaptability and rubber production (Wuyun et al., 2017). Conversely, in *H. brasiliensis*, the regulation of rubber biosynthesis is closely linked to the expression of genes involved in stress responses and defense mechanisms, as evidenced by the high abundance of transcripts related to these functions in latex (Chow et al., 2007). This indicates that while both species have evolved sophisticated regulatory systems to optimize rubber production, the specific pathways and gene families involved differ significantly.

6 Case Studies

6.1 Genetic modification in *Eucommia ulmoides*

Genetic modification in *Eucommia ulmoides* has been a focal point of research due to its unique rubber biosynthesis pathway. The high-quality haploid genome assembly of *E. ulmoides* has provided significant insights into its genetic structure and potential for genetic engineering. The genome assembly, achieved through PacBio and Hi-C technologies, revealed a more primitive rubber biosynthesis pathway that relies on the methylerythritol-phosphate (MEP) pathway rather than the mevalonate pathway, which is predominant in *Hevea brasiliensis* (Li et al., 2020) (Figure 2). This discovery opens avenues for genetic modifications aimed at enhancing rubber yield and quality by targeting specific genes involved in the MEP pathway. Additionally, the identification of long non-coding RNAs (lncRNAs) and their regulatory roles in rubber biosynthesis further underscores the potential for genetic interventions. These lncRNAs and transcripts of uncertain coding potential (TUCPs) regulate key genes involved in the biosynthesis process, suggesting that genetic modifications could be directed to optimize these regulatory networks (Liu et al., 2018).

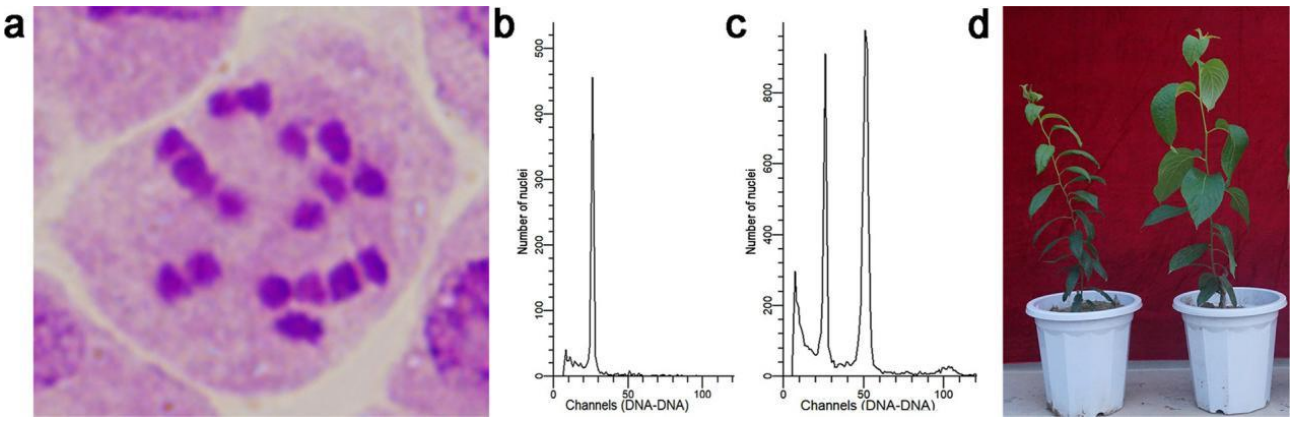

Figure 2 A Somatic chromosome number of the haploids (2n = x = 17). b Ploidy levels obtained from 3-week-old first leaf samples from haploid plants by flow cytometric analysis. c Ploidy levels obtained from 3-week-old first leaf samples from a mixture of haploid and diploid plants by flow cytometric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

6.2 Breeding efforts in *Hevea brasiliensis*

Breeding efforts in *Hevea brasiliensis* have traditionally focused on improving rubber yield, disease resistance, and stress tolerance. Unlike *E. ulmoides*, *H. brasiliensis* synthesizes cis-polyisoprene via the mevalonate pathway. The genetic basis for this pathway has been well-studied, and breeding programs have leveraged this knowledge to develop high-yielding and disease-resistant clones. The genome of *H. brasiliensis* has been extensively mapped, revealing key genes involved in rubber biosynthesis and stress responses. These genes have been targeted in breeding programs to enhance the overall productivity and resilience of rubber trees. Comparative studies have shown that while *E. ulmoides* relies on the MEP pathway, *H. brasiliensis* has evolved a distinct set of genes for cis-polyisoprene synthesis, providing a rich genetic resource for breeding efforts (Wuyun et al., 2017).

6.3 Comparative outcomes of biosynthesis pathway modifications

The comparative outcomes of biosynthesis pathway modifications in *Eucommia ulmoides* and *Hevea brasiliensis* highlight the distinct evolutionary paths these species have taken. *E. ulmoides*, with its reliance on the MEP pathway, offers a unique model for studying rubber biosynthesis. Genetic modifications targeting the MEP pathway in *E. ulmoides* could lead to significant improvements in rubber yield and quality, leveraging its unique genetic makeup (Liu et al., 2018; Li et al., 2020). On the other hand, *H. brasiliensis*, with its well-characterized mevalonate pathway, continues to benefit from traditional breeding and genetic engineering efforts aimed at enhancing cis-polyisoprene production. The independent expansion of farnesyl diphosphate synthases (FPSs) and rubber elongation factor/small rubber particle protein gene families in *E. ulmoides* compared to *H. brasiliensis* underscores the divergent evolutionary strategies these species have adopted for rubber biosynthesis (Wuyun et al., 2017). This comparative analysis not only provides insights into the fundamental biology of rubber production but also informs targeted genetic and breeding strategies to optimize rubber yield and quality in both species.

7 Environmental and Ecological Factors Influencing Rubber Biosynthesis

7.1 Influence of climatic conditions

Climatic conditions play a crucial role in the biosynthesis of rubber in both *Eucommia ulmoides* and *Hevea brasiliensis*. The rubber tree (*Hevea brasiliensis*) thrives in tropical climates with high humidity and consistent rainfall, which are essential for optimal latex production. These conditions help maintain the physiological processes necessary for rubber biosynthesis, including the activity of enzymes like farnesyl pyrophosphate synthase (FPS) that are critical for the formation of polyisoprenoids (Chuntai et al., 2017). On the other hand, *Eucommia ulmoides*, also known as the hardy rubber tree, is more adaptable to a wider range of climatic conditions, including temperate zones. This adaptability is partly due to its unique biosynthetic pathway that relies on the methylerythritol-phosphate (MEP) pathway rather than the mevalonate pathway, which is predominant in *Hevea brasiliensis* (Wuyun et al., 2017; Li et al., 2020).

7.2 Soil and nutrient impacts on biosynthesis

Soil composition and nutrient availability significantly impact rubber biosynthesis in both species. *Hevea brasiliensis* is often cultivated in acidic soils, which can contain high levels of aluminum. The rubber tree has developed mechanisms to tolerate these conditions, such as the expression of aluminum-activated malate transporter (ALMT) genes that help in aluminum detoxification, thereby supporting healthy growth and latex production (Ma et al., 2020). In contrast, *Eucommia ulmoides* shows a different set of adaptations to its soil environment. The presence of specific long non-coding RNAs (lncRNAs) and differentially expressed genes involved in cellular processes like cell wall formation and growth suggests that *E. ulmoides* has evolved to efficiently utilize available nutrients for rubber biosynthesis (Liu et al., 2018).

7.3 Adaptations of *Eucommia ulmoides* and *Hevea brasiliensis* to their environments

Eucommia ulmoides and *Hevea brasiliensis* have developed distinct adaptations to their respective environments, which influence their rubber biosynthesis pathways. *Eucommia ulmoides*, with its high expression levels of genes involved in stress responses and secondary metabolite biosynthesis, exhibits considerable environmental adaptability. This adaptability is reflected in its ability to synthesize trans-polyisoprene via expanded farnesyl diphosphate synthase (FPS) gene families, which are distinct from those in *Hevea brasiliensis* (Wuyun et al., 2017). Additionally, the genome of *E. ulmoides* has undergone unique evolutionary events, such as a whole-genome duplication, which have contributed to its robust rubber biosynthesis capabilities (Li et al., 2020).

Hevea brasiliensis, on the other hand, has specialized in producing cis-polyisoprene and has developed specific physiological and biochemical mechanisms to thrive in tropical environments. The expression of FPS genes in various tissues, particularly in response to environmental stresses like bark tapping and hormonal treatments, highlights the tree's adaptation to its ecological niche (Chuntai et al., 2017). These adaptations ensure efficient rubber production even under varying environmental conditions, making *Hevea brasiliensis* a dominant source of natural rubber.

In summary, while both *Eucommia ulmoides* and *Hevea brasiliensis* produce natural rubber, their biosynthesis pathways and environmental adaptations are shaped by their unique ecological contexts. Understanding these factors can provide insights into improving rubber yield and quality through targeted genetic and environmental management strategies.

8 Industrial Applications and Economic Potential

8.1 Utilization of rubber from *Eucommia ulmoides*

Eucommia ulmoides rubber (EUR) has garnered significant attention due to its unique properties and potential applications. Unlike the traditional natural rubber from *Hevea brasiliensis*, which is composed of cis-1,4-polyisoprene, EUR is primarily made up of trans-1,4-polyisoprene. This structural difference imparts EUR with a dual nature, exhibiting characteristics of both rubber and plastic, making it a novel material in various industries. The extraction, structure, and physicochemical properties of EUR have been extensively studied, revealing its potential in environmental, agricultural, engineering, and biomedical fields (Wei et al., 2021). The high-quality genome assembly of *E. ulmoides* has further facilitated research into its rubber biosynthesis pathways, enabling genetic engineering efforts to enhance its industrial applications (Wuyun et al., 2017; Li et al., 2020).

8.2 Market demand for natural rubber

The global demand for natural rubber is substantial, driven by its extensive use in manufacturing over 50,000 products, including tires and medical gloves (Cherian et al., 2019) (Figure 3). *Hevea brasiliensis* remains the primary source of natural rubber, but its production is vulnerable to diseases and climatic changes, prompting the search for alternative sources. The unique properties of EUR position it as a promising alternative, potentially alleviating some of the supply pressures on *H. brasiliensis*. The increasing interest in EUR is also fueled by its potential to meet specific industrial needs that traditional natural rubber cannot, due to its distinct trans-polyisoprene composition (Chow et al., 2007; Wei et al., 2021).

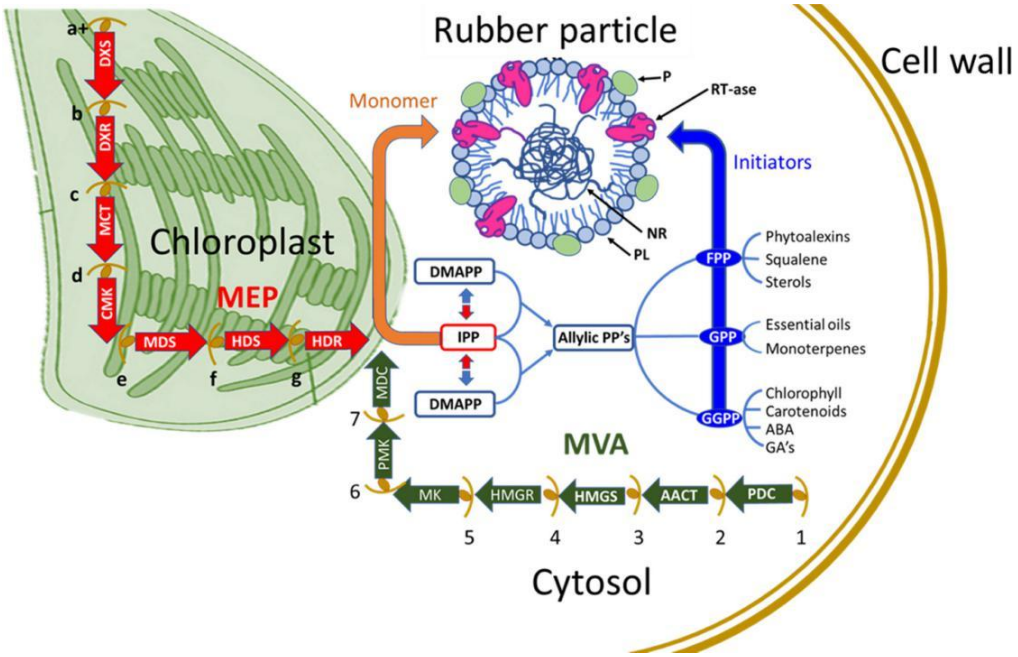

Figure 3 The metabolic route map for natural rubber (cis-1,4-polyisoprene) biosynthesis in plants, including the pathways for substrate synthesis, and their locations (Adopted from Cherian et al., 2019)

Image caption: Isopentenyl pyrophosphate (IPP), the monomeric subunit for rubber biosynthesis (orange arrow) is synthesized by two pathways, the mevalonic acid pathway (MVA, cytosolic, green arrows and numerals) and the methylerythritol pathway (MEP, plastidic, red arrows and lower case letters) from acetyl-CoA or glyceraldehyde-3-phosphate and pyruvate, respectively. IPP and its stereoisomer dimethylallyl pyrophosphate (DMAPP) condense to form several allylic pyrophosphates (APPs), namely geranyl pyrophosphate (GPP, C10), farnesyl pyrophosphate (FPP, C15) and geranyl geranyl pyrophosphate (GGPP, C20). These APPs can be used as rubber chain initiators (blue arrow), FPP being the most common initiator, and are also the building blocks for terpenes such as chlorophyll, sterols, plant growth regulators, essential oils and so forth. Natural rubber biosynthesis is catalysed by rubber transferase complexes (magenta) bound to the proteolipid uni-lamella membrane (light blue) of cytosolic rubber particles, and rubber is compartmentalized to the rubber particle interior. Key: MVA enzymes: PDC, pyruvate dehydrogenase complex; AACT, acetyl coenzyme A acetyltransferase; HMGS, hydroxymethylglutaryl coenzyme A synthase; HMGR, hydroxymethylglutaryl coenzyme A reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; MDC, diphosphomevalonate decarboxylase. MVA substrates: 1. pyruvate; 2. acetyl coenzyme A; 3. acetoacetyl coenzyme A; 4. hydroxymethylglutaryl coenzyme A; 5. mevalonate; 6. phosphomevalonate; 7. diphosphomevalonate. MEP enzymes: 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. MEP substrates: a+, pyruvate and D-glyceraldehyde 3-phosphate; b. 1-deoxy-D-xylulose 5-phosphate; c. 2-C-methyl-D-erythritol 4-phosphate; d. 4-(cytidine 5/-diphospho)-2-C-methyl-D-erythritol; e. 2-phospho-4-(cytidine 5/-diphospho)-2-C-methyl-D- erythritol; f. 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; g. (E)-4-hydroxy-3-methylbut-2-enyl diphosphate. RP, rubber particle; RT-ase, rubber transferase complex; P, non-RT-ase rubber particle-associated proteins; complexes; PL, proteolipid unilamella membrane; NR, natural rubber polymers (Adopted from Cherian et al., 2019)

8.3 Economic impact of enhanced biosynthesis pathways

Advancements in understanding and manipulating the biosynthesis pathways of rubber in both *E. ulmoides* and *H. brasiliensis* have significant economic implications. The identification of key genes and enzymes involved in rubber biosynthesis, such as the rubber elongation factor and small rubber particle protein, has opened avenues for metabolic engineering to enhance rubber yield and quality (Chow et al., 2007; Liu et al., 2018). The high-quality genome assemblies and transcriptome analyses of these species provide a robust foundation for genetic modifications aimed at increasing rubber production efficiency (Wuyun et al., 2017; Li et al., 2020). Enhanced biosynthesis pathways not only promise to boost the economic viability of EUR but also ensure a more stable and diversified supply of natural rubber, mitigating the risks associated with over-reliance on a single source (Cherian et al., 2019).

9 Challenges and Future Directions in Rubber Research

9.1 Technical challenges in biosynthesis pathway modification

Modifying the biosynthesis pathways of rubber in both *Eucommia ulmoides* and *Hevea brasiliensis* presents several technical challenges. One significant challenge is the complexity of the rubber biosynthesis pathways themselves. In *E. ulmoides*, the methylerythritol-phosphate (MEP) pathway is predominantly used for isoprenyl diphosphate synthesis, whereas *H. brasiliensis* primarily utilizes the mevalonate (MVA) pathway (Chow et al., 2011; Li et al., 2020). This difference necessitates distinct approaches for pathway modification in each species. Additionally, the regulation of these pathways involves numerous genes and enzymes, making targeted modifications difficult. For instance, the role of long non-coding RNAs (lncRNAs) in regulating rubber biosynthesis in *E. ulmoides* adds another layer of complexity (Liu et al., 2018). Furthermore, the presence of transposable elements (TEs) and their derived small interfering RNAs (siRNAs) in *H. brasiliensis* can interfere with gene expression, complicating genetic modifications (Wu et al., 2020).

9.2 Opportunities for genetic engineering

Despite these challenges, there are significant opportunities for genetic engineering to enhance rubber production. Advances in genome sequencing and assembly have provided high-quality genomic resources for both *E. ulmoides* and *H. brasiliensis*, facilitating the identification of key genes involved in rubber biosynthesis (Lau et al., 2016; Wuyun et al., 2017; Li et al., 2020). Genetic engineering can target these genes to improve rubber yield and quality. For example, the expansion of rubber biosynthesis-related gene families in *H. brasiliensis* suggests potential targets for enhancing latex production (Lau et al., 2016). Additionally, the identification of differentially expressed lncRNAs and microRNAs in *H. brasiliensis* offers new avenues for manipulating gene expression to increase rubber yield (Li et al., 2022). The development of transgenic rubber trees with enhanced biosynthetic pathways could significantly boost rubber production (Chow et al., 2011).

9.3 Future research directions in rubber biosynthesis

Future research in rubber biosynthesis should focus on several key areas. First, a deeper understanding of the regulatory networks controlling rubber biosynthesis is essential. This includes studying the roles of lncRNAs, microRNAs, and TEs in gene regulation (Liu et al., 2018; Wu et al., 2020; Li et al., 2022). Second, research should aim to elucidate the final stages of rubber elongation, which remain poorly understood (Wu et al., 2020). Third, comparative studies between *E. ulmoides* and *H. brasiliensis* can provide insights into the evolution of rubber biosynthesis pathways and identify common regulatory mechanisms (Wuyun et al., 2017). Finally, integrating genomic, transcriptomic, and epigenetic data will be crucial for developing effective genetic engineering strategies to enhance rubber production (Chow et al., 2007; Lau et al., 2016; Li et al., 2020). By addressing these research directions, we can improve our understanding of rubber biosynthesis and develop innovative approaches to meet the growing demand for natural rubber.

10 Concluding Remarks

This comparative study of rubber biosynthesis pathways in *Eucommia ulmoides* and *Hevea brasiliensis* has revealed significant differences and similarities in their genetic and biochemical mechanisms. The high-quality genome assembly of *E. ulmoides* has provided new insights into its evolution and rubber biosynthesis, highlighting the reliance on the methylerythritol-phosphate (MEP) pathway for isoprenyl diphosphate synthesis, which is predominantly active in trans-polyisoprene-containing leaves and central peels. In contrast, *H. brasiliensis* primarily utilizes the mevalonate (MVA) pathway for cis-polyisoprene biosynthesis, with evidence suggesting a potential role for the MEP pathway as well. Additionally, the expansion of gene families related to rubber biosynthesis in *H. brasiliensis* has been identified as a key factor contributing to its high latex yield. Differential expression of long noncoding RNAs (lncRNAs) and microRNAs between self-rooting juvenile clones and donor clones of *H. brasiliensis* further unveils the molecular regulation underlying increased rubber yield.

The insights gained from this study have several potential applications. The high-quality genome assembly of *E. ulmoides* can facilitate genetic engineering efforts to enhance its industrial and medicinal uses. Understanding the distinct biosynthesis pathways in *E. ulmoides* and *H. brasiliensis* can lead to the development of transgenic rubber

trees with optimized rubber production by manipulating the MEP and MVA pathways. The identification of key regulatory genes and noncoding RNAs involved in rubber biosynthesis can be leveraged to improve rubber yield and quality through targeted breeding programs and biotechnological interventions. Moreover, the comparative genomic data can aid in the discovery of novel genes and pathways that could be exploited for synthetic biology applications to produce rubber and other valuable isoprenoids in microbial systems.

The future of rubber biosynthesis research holds promising potential for both fundamental and applied sciences. Continued efforts in genome sequencing and functional genomics will be crucial to uncover the complex regulatory networks governing rubber biosynthesis in different species. Integrating multi-omics approaches, including transcriptomics, proteomics, and metabolomics, will provide a holistic understanding of the biosynthetic pathways and their regulation. Advances in CRISPR/Cas9 and other gene-editing technologies offer exciting opportunities to precisely modify key genes and pathways to enhance rubber production. Collaborative research across disciplines, including plant biology, genetics, biochemistry, and bioengineering, will be essential to translate these scientific insights into practical applications, ultimately leading to sustainable and efficient rubber production systems.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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