

## Feature Review

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# Advances in Genetic Regulation of Latex Biosynthesis and Wounding Response in Rubber Tree (*Hevea brasiliensis*)

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**Abstract** This study summarizes the core synthetic process of latex, including the formation of IPP precursors through the MVA/MEP pathway, as well as the extension process of the cis-polyisoprene chain under the action of HMGR, FPS, CPT and rubber granular related proteins. It also sorts out the expression characteristics of key enzyme genes and points out the rate-limiting steps that may affect the synthetic efficiency of latex. The regulatory effects of calcium signaling, MAPK cascade reaction, and hormone signals such as jasmonic acid and ethylene on latex synthesis under cut conditions were discussed. The molecular mechanisms of transcription factors such as MYC, JAZ, and ERF in latex regeneration, wound defense, and latex fluidity regulation were explained. The genetic differences affecting high-yield traits were reviewed. The interaction between the latex synthesis pathway and the defense pathway was discussed, and feasible strategies for increasing latex production, such as genetic engineering, metabolic engineering, hormone treatment and optimization of the rubber tapping system, were summarized. This research aims to provide a theoretical basis for the application of biotechnology in high-yield breeding and sustainable production of rubber trees.

**Keywords** Rubber tree (*Hevea brasiliensis*); Latex biosynthesis; Wounding and latex flow response; Signal transduction and transcriptional regulation; Multi-omics and molecular breeding

## 1 Introduction

Natural rubber (NR) is particularly important in many industries due to its good elasticity, strong wear resistance and impact resistance. Nowadays it is often used in tires, medical equipment, aviation materials, etc. (Yamaguchi et al., 2020; He et al., 2024). At present, approximately 99% of the world's natural rubber comes from rubber trees in Brazil, with an annual output of over 14 million tons, mainly concentrated in tropical regions such as Southeast Asia, Africa and South America (Cheng et al., 2023; Li et al., 2025). With the increasingly rapid development of some emerging economies, the global demand for natural rubber is also constantly increasing, especially countries such as China, which have now become major consumers and importers (Yamaguchi et al., 2020). However, the production of natural rubber mostly relies on small-scale farmers and is greatly affected by climate, pests and diseases, and changes in market prices. Therefore, how to increase rubber output and make production more stable is a concern for all (Li et al., 2025).

The rubber tree is a perennial tropical tree. The laticifers system within its body is the main site for the synthesis of natural rubber. Laticifers cells differentiate from the cambium and have a very complex structure, like a net. Latex is actually the cytoplasm of laticifers cells, which contains approximately 30% to 50% natural rubber (Yamaguchi et al., 2020). The synthesis of rubber uses IPP (isopentenyl pyrophosphate) as a precursor and is regulated by a variety of key enzymes and genes (He et al., 2024). The quantity of laticifers and their activity level will directly affect the rubber output. Rubber tapping (tapping) is the most common method of rubber harvesting nowadays, but this process causes mechanical damage to the tree and triggers a series of physiological responses, such as changes in hormone signals like ethylene and jasmonic acid, thereby affecting latex regeneration and the defense response of the tree (Flórez-Velasco et al., 2024; Li et al., 2025). Furthermore, some genes that are particularly highly expressed in laticifers, as well as the transcriptional and post-transcriptional regulatory mechanisms regulated after cuts, are also very important for rubber synthesis and stress resistance response (Leclercq et al., 2020).

This study mainly collates the genetic regulatory progress of rubber trees in natural rubber biosynthesis and cut response in recent years, with a focus on key genes, signaling pathways and transcription factors, and discusses their mechanisms of action in increasing latex yield and enhancing stress resistance. This study aims to integrate the achievements of multi-omics, molecular biology and physiology to provide new ideas and foundations for high-yield and stress-resistant breeding of rubber trees.

## 2 Latex Biosynthesis Pathways in Rubber Tree

### 2.1 Mevalonate (MVA) and MEP pathways involved in isoprenoid precursor formation

In the latex of rubber trees, isopentenyl pyrophosphate (IPP) is mainly synthesized through the MVA pathway in the cytoplasm. However, the MEP pathway in chloroplasts can also provide some IPP. The MVA pathway starts with acetyl-CoA and undergoes multiple enzymatic reactions to generate IPP. The MEP pathway, on the other hand, uses pyruvate and glyceraldehyde 3-phosphate as starting points to synthesize IPP. Research has found that the MVA pathway is the main source of rubber biosynthesis, while the MEP pathway is more often used in the production of other compounds such as carotenoids. However, in some cases, the MEP pathway may also provide some precursors for rubber synthesis (Deng et al., 2018). These two pathways are located differently within the cell, but some intermediates like IPP can move between the cytoplasm and the plastid. Their specific distribution may be related to the variety of rubber trees and the content of carotenoids in latex (Long et al., 2021; Xu et al., 2025).

### 2.2 Rubber particle formation and polymerization mechanisms

Rubber particles are a unique structure in latex cells, used for the synthesis and storage of natural rubber. At its center is the hydrophobic cis-1,4-polyisoprene, surrounded by a monolayer lipid membrane, within which there are also many proteins embedded. There are several key enzymes in rubber biosynthesis, such as CPT (cis-prenyltransferase), REF (rubber elongation factor), and SRPP (small rubber granule protein). CPT is responsible for polymerizing IPP, and FPP (farnesyl pyrophosphate) serves as the initiator of the reaction. SRPP and REF, on the other hand, facilitate the smooth progress of the polymerization reaction and influence the length and efficiency of the rubber chain. The formation of rubber particles and rubber synthesis occur simultaneously. If CPT or its activating factors are absent, rubber particles cannot form normally and rubber synthesis will also be blocked (Yamashita and Takahashi, 2020; Fan et al., 2022; Nie et al., 2022).

### 2.3 Rate-limiting steps and metabolic bottlenecks in latex production

The output of latex is often subject to various metabolic constraints. Firstly, HMGR in the MVA pathway is a rate-limiting step. The expression level and activity of this enzyme will directly affect the generation rate of IPP (Deng et al., 2018; Wu et al., 2018). In addition, the expression levels of FPS (FPP synthase) and CPT are also related to the rubber synthesis capacity. If the FPS is reduced, the content of FPP will decrease, and then it will affect the formation of rubber particles and the polymerization of rubber molecules (Nie et al., 2022). In addition to these enzymes, substrate supply (such as the transport and metabolism of sucrose), energy supply (such as the TCA cycle and glycolysis), and the fluidity of latex (affected by moisture, osmotic pressure and latex blockage) will also become important limiting factors (Fan et al., 2022; Qin et al., 2022; Qin et al., 2023). Some hormones, such as ethylene, jasmonic acid and brassinolide, can regulate the expression of related genes, thereby alleviating some metabolic bottlenecks and increasing latex yield (Figure 1) (Deng et al., 2018; Guo et al., 2023; Flórez-Velasco et al., 2024).

## 3 Key Genes and Enzymes Regulating Latex Biosynthesis

### 3.1 Cis-prenyltransferases, rubber transferases, and isoprenoid pathway enzymes

The synthesis of latex mainly relies on the generation of IPP (isoprene unit) and further polymerization. Rubber trees mainly synthesize IPP through the MVA pathway in the cytoplasm, and then these IPPs are polymerized on the surface of latex particles into cis-1,4-polyisoprene, which is natural rubber. During this process, many enzymes will play key roles, including CPT (cis-terpenyltransferase), rubber transferase, and several major enzymes in the MVA pathway, such as HMGS, HMGR, MVD and FPS (Jayashree et al., 2018; Wu et al., 2018; Bakar and Othman, 2022). In rubber trees, there are many members of the CPT family, among which CPT2 has

been proven to be capable of long-chain polymerization. In addition to these enzymes, REF (rubber elongation factor) and SRPP (small rubber granule protein) are also very important. They are proteins on the latex particle membrane, which not only participate in polymerization but also affect the stability and mode of action of the particles.

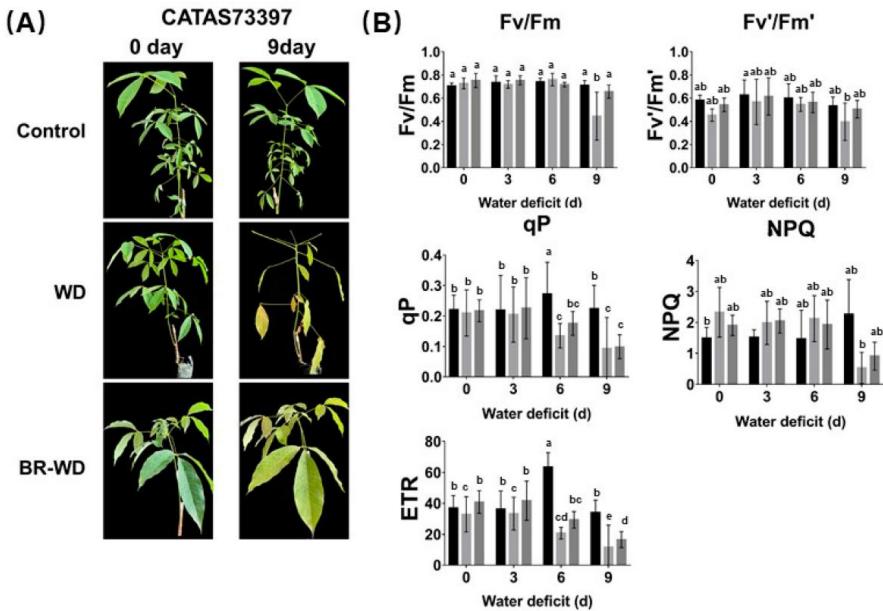


Figure 1 Effects of BR treatment on phenotype and photosynthesis of rubber trees under water deficit (Adopted from Guo et al., 2023)

### 3.2 Gene families involved in rubber particle stability and function

In the genome of rubber trees, the REF/SRPP family is significantly amplified, and there are many latex-specific types. REF1 is the main protein on large particles, while SRPP is more abundant on small particles. These proteins interact with enzymes such as CPT, thereby affecting the polymerization of rubber and the stability of particles. In addition to REF and SRPP, proteins such as RPMPs (latex particle-related proteins), ABC transporters, and ACBP (acyl-CoA binding proteins) also play roles in latex metabolism and particle function (Nie et al., 2018). The amplification and high expression of these gene families are regarded as one of the important genetic bases for rubber trees to achieve high latex yields.

### 3.3 Expression patterns associated with high- and low-yielding cultivars

Among high-yield rubber tree varieties, the expression levels of some key genes were significantly higher, such as CPT, REF, SRPP, HMGS, HMGR, MVD and FPS (Wu et al., 2018; Bakar and Othman, 2022). For instance, the mRNA content of the REF gene in latex cells of high-yield varieties can be five times that of low-yield varieties, and it is positively correlated with yield. Similarly, overexpression of HMGR1 can also significantly increase latex yield (Jayashree et al., 2018). Meanwhile, hormone treatments such as ethylene and methyljasmonic acid, as well as mechanical stimulation caused by rubber tapping, can induce the expression of these genes, thereby promoting latex synthesis (Zhu et al., 2018; Chao et al., 2019). The expression differences among different varieties or genotypes also indicate that the formation of latex yield is closely related to genetic regulation (Bakar and Othman, 2022; Cheng et al., 2023).

## 4 Wounding Response Mechanisms in Rubber Tree

### 4.1 Signal transduction pathways activated by tapping or mechanical stress

The mechanical damage caused by rubber tapping will first trigger the signal transduction response of the tree, among which the MAPK cascade is one of the earliest activated pathways. Genome-wide analysis revealed that there were a total of 20 HbMPK, 13 HbMPKK and 167 HbMAPKKK genes in the rubber tree. The expression differences of these genes in different tissues and under various stress conditions are quite obvious. After rubber tapping, the expression of some MAPK members (such as HbMPK8, HbMPK12, HbMPK19, etc.) increased in

latex or cut tissue, indicating that they may have a direct relationship with latex cell development and rubber synthesis. In addition, the CBL-CIPK network in the calcium signaling pathway also undergoes significant changes after rubber tapping or ethylene stimulation. This network can integrate multiple signals and participate in latex flow and stress responses (Xiao et al., 2022; Huang et al., 2025).

#### 4.2 Hormonal response networks (ethylene, jasmonate, ABA)

The "flow loss" process caused by rubber tapping is jointly regulated by multiple plant hormones. Jasmonic acid (JA) is a very important signal among them. It participates in the flow injury reaction and can also promote the differentiation of latex cells and the expression of genes related to rubber synthesis. The JAZ protein family is an inhibitor of JA signaling. After rubber tapping and exogenous JA treatment, the expression of JAZ members would increase and form regulatory modules with MYC-like transcription factors, jointly regulating the changes of downstream genes (Zhai et al., 2018; Chao et al., 2019). Ethylene (ET) also functions through the ERF family, thereby influencing latex flow and defense responses. Rubber tapping, ethylene and jasmonic acid can jointly induce the expression of ERF-IX genes, while ethylene inhibitors block this process, indicating that this expression depends on ethylene signaling. Other hormones, such as ABA (abscisic acid), also interact with JA and ET signals to participate in regulating injury current and defense responses, making the regulatory process more refined (Mu et al., 2025).

#### 4.3 Defense-related genes and their interactions with latex biosynthesis

After rubber tapping, rubber trees also activate a large number of defense-related genes, including antioxidant enzymes, PR proteins (such as PR1, chitinase), and various secondary metabolism-related enzymes. These genes not only participate in wound repair and disease resistance responses, but also are associated with the latex biosynthesis pathway. For instance, rubber tapping can promote the expression of key enzymes for rubber synthesis such as REF, SRPP, and FPS1, and also upregulate genes related to energy metabolism to support latex regeneration and the continuous synthesis of rubber (Nie et al., 2022). Furthermore, some transcription factors, such as MYC, ERF and bHLH, are involved in regulating the balance between defense and latex synthesis, enabling the tree to maintain a certain latex yield under defense pressure (Zhai et al., 2018; He et al., 2024).

### 5 Multi-Omics Approaches to Understanding Latex Production and Wound Response

#### 5.1 Genomic and transcriptomic insights into regulatory gene clusters

High-quality telomere to telomere (T2T) genomes enable researchers to more comprehensively identify genes related to latex biosynthesis and clearly observe the expression of different alleles. By combining transcriptome and metabolome data, the study further confirmed that the MVA pathway is the main carbon source for the rapid regeneration of latex. Meanwhile, jasmonic acid (JA) can activate transcription factors such as MYC2, thereby increasing the expression of some key enzymes (such as MVK1) and enhancing the synthetic capacity of latex. In addition, JA also works in conjunction with hormone signals such as auxin (IAA) and ethylene (ET) to regulate latex production. A variety of related transcription factors, such as JAZ, MYC2, AP2/ERF1B, all increase in expression after mechanical injury, indicating that the regulation of latex yield involves a complex hormonal interaction network (Bakar and Othman, 2022; Huang et al., 2025; Li et al., 2025).

#### 5.2 Proteomic and metabolomic analyses of latex-producing tissues

Proteomics research has found that there are many key proteins in latex, such as REF and SRPP, which not only participate in the synthesis of latex but also play a role in responding to stress. The combined analysis of proteomics and metabolomics further revealed that lipid metabolism, glucose metabolism and antioxidation-related proteins would undergo significant changes under rubber tapping or hormonal stimulation, indicating that the synthesis process of latex is interrelated with the defense response. In addition, members of the ACBP family are also important in latex and lipid metabolism, and their expression is significantly upregulated after mechanical injury or JA treatment (Nie et al., 2018; Habib and Ismail, 2020; Leclercq et al., 2020).

#### 5.3 Systems biology models linking wounding signals with metabolic pathways

Systems biology combines multiple omics data to construct a regulatory model between wound signaling and latex synthesis. Research has found that JA signaling directly regulates key enzymes in the MVA pathway through

MYC2, and also interacts with hormone signals such as IAA and ET, forming a multi-level regulatory network. The MAPK cascade and the CBL-CIPK calcium signaling pathway have also been demonstrated to be involved in latex synthesis and wound response. Furthermore, the expression of small RNAs in latex tissues is highly active, and they can affect multiple links such as glucose metabolism and rubber synthesis, indicating that the gene regulation in latex tissues is multi-level (Xiao et al., 2022; Huang et al., 2025; Li et al., 2025).

## 6 Biotechnological Strategies to Enhance Latex Yield

### 6.1 Genetic engineering and transcription factor manipulation

In recent years, significant progress has been made in the improvement of latex biosynthesis from rubber trees through genetic engineering and the regulation of transcription factors. Multi-omics studies have shown that the two transcription factor families, bHLH and ERF, play a major role in latex biosynthesis and flow injury reactions. Transcription factors of bHLH type (such as Hb\_MYC2-1, Hb\_MYC2-2, Hb\_bHLH1, Hb\_bHLH2) not only affect the differentiation of latex cells, but also directly increase the expression of rubber synthesis genes such as SRPP and REF. Thereby increasing latex production (Figure 2) (Yamaguchi et al., 2020; He et al., 2024). In addition, HbWRKY83 in the WRKY family can activate genes related to JA, ethylene, and flow reaction, promote latex synthesis, and also help the tree improve stress resistance (Kang et al., 2020). GWAS studies also found that variations in genes related to glucose transport, glucose metabolism, and ethylene signaling were highly correlated with high-yield traits, and the occurrence frequencies of these key alleles significantly increased during breeding (Bakar and Othman, 2022; Cheng et al., 2023). These results provide important theoretical basis and candidate genes for regulating and increasing latex yield through genetic engineering or transcription factors.

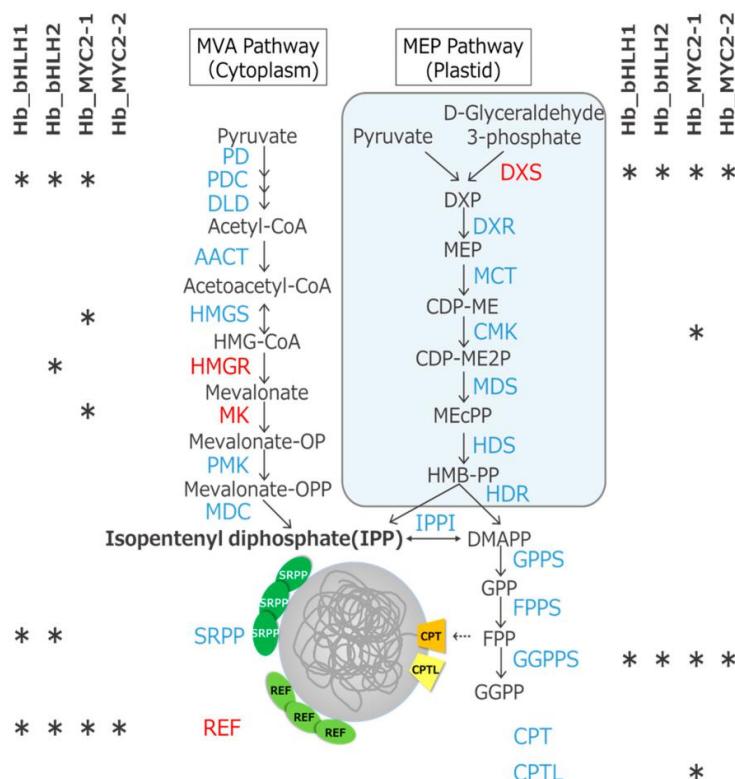


Figure 2 Candidate genes targeted by four bHLH-type TFs in the MVA, MEP, and rubber biosynthetic pathways (Adopted from Yamaguchi et al., 2020)

### 6.2 CRISPR/Cas-based genome editing and potential applications

The CRISPR/Cas system is now capable of achieving relatively efficient gene editing in rubber trees. Studies have shown that when sgRNA is driven by the U6 promoter of the rubber tree itself, an editing rate of approximately 8.5% to 25% can be achieved in protoplast and callus. Common mutations include base deletion, insertion and substitution (Dai et al., 2021). After knocking out genes with CRISPR/Cas9, researchers have obtained some expected phenotypes, such as albinism phenotypes, and these traits can also be stably passed on to the next

generation. Meanwhile, DNA-free editing was also achieved by direct delivery of RNP (ribonucleoprotein), which brought a new approach to molecular breeding and functional gene research of rubber trees (Fan et al., 2020). Although current CRISPR research mainly focuses on traits such as flowering time, with the gradual improvement of the regeneration system, there will be opportunities in the future to edit key genes such as latex synthesis and stress resistance, accelerating the breeding process of high-yield rubber trees (Dai et al., 2021; Cardi et al., 2023).

### 6.3 Latex production improvement through optimized tapping and hormone treatments

Rubber tapping methods and exogenous hormone treatment (such as ethylene, jasmonic acid, abscisic acid, gibberellin, etc.) are also effective management measures to increase latex production. The use of ethylene releasers (such as ethephon) can prolong the flow time of latex, promote latex regeneration, and usually increase the yield by 1.5 to 2 times (Purwaningrum et al., 2019). Hormone treatment promotes the increased expression of genes related to glucose metabolism, sucrose transport, rubber synthesis (such as SRPP, REF, CPT, etc.) and aquaporin (such as HbPIP2;1, HbTIP1;1), helping to maintain the turgor pressure and fluidity of latex cells (Wu et al., 2018; Qin et al., 2023; Flórez-Velasco et al., 2024). Meanwhile, by adjusting the rubber tapping frequency, cutting surface length and Angle (for example, S/4d3 combined with ethylene stimulation), the yield per unit area can also be significantly increased without increasing the tree pressure (Purwaningrum et al., 2019). In addition, if reasonable irrigation and fertilization management is combined with hormone treatment, it can further increase the output of latex and dry rubber.

## 7 Case Study: Transcriptomic-Guided Enhancement of Latex Yield in Elite Rubber Tree Clone

### 7.1 Background: low-yield challenges and research objectives

Rubber trees (*Hevea brasiliensis*), as the main source of natural rubber, have long faced the problem of limited increase in latex production. Although traditional breeding has increased latex production by approximately six times over the past century or so, the genetic basis for high yields remains unclear. Moreover, some well-performing clones still experience significant fluctuations or low yields in actual production. To break through this limitation, it is necessary to figure out how high-yield traits are regulated at the molecular level and use this information to guide molecular breeding and precision improvement (Wu et al., 2018; Bakar and Othman, 2022; Cheng et al., 2023).

### 7.2 Omics-driven identification of yield-enhancing genes and regulatory targets

In recent years, transcriptome and multi-omics technologies have facilitated the rapid screening of high-yield-related genes. RNA sequencing of latex, bark and leaves of high-yield and low-yield clones revealed that genes such as AACT, MVD, SRPP and REF were significantly upregulated in high-yield varieties and were closely related to yield (Wu et al., 2018). Meanwhile, ethylene response factor (ERF), sugar transport genes, and genes involved in ethylene synthesis and signal transduction are also strongly correlated with latex yield (Bakar and Othman, 2022). The GWAS study further identified 155 marker sites related to latex yield, involving 326 candidate genes, providing a large number of available targets for molecular breeding (Cheng et al., 2023).

### 7.3 Implementation of wounding-responsive gene manipulation and resulting yield improvement

Rubber tapping (trauma) and exogenous hormone treatment (such as ethylene and jasmonic acid) can significantly induce the expression of genes related to latex metabolism, promoting latex regeneration and flow. Studies have shown that continuous rubber tapping promotes the accumulation of JA (jasmonic acid), thereby up-regulating the expression of key enzymes such as MVK1 in the MVA pathway and enhancing the synthetic ability of latex (Li et al., 2025). Ethylene treatment mainly increases latex production by promoting sugar flow and carbon metabolism. In high-yield clones, the expression levels of these related genes are positively correlated with the number of rubber tapping times and the final yield. After regulating the expression of these genes, the latex yield could also be significantly increased (Wu et al., 2018; Bakar and Othman, 2022). Furthermore, some transcription factors, such as the bHLH and MYC families, play key roles in the regulation of latex biosynthesis by trauma and hormone signaling, and thus have also become important research directions for genetic engineering improvement (Zhai et al., 2018; Yamaguchi et al., 2020).

## **8 Challenges and Future Directions**

### **8.1 Limitations in genetic transformation and long breeding cycles**

The rubber tree (*Hevea brasiliensis*) is a perennial woody crop with strong heterogeneity and a relatively complex genomic structure. These characteristics make the cycle of traditional breeding very long, usually 25 to 30 years, which significantly limits the rapid cultivation of new varieties with high yield and stress resistance. Although molecular breeding techniques such as MAS, GWAS and GS have been applied in recent years and have brought the possibility of accelerating breeding, the genetic transformation efficiency of rubber trees is still not high, the tissue culture system is difficult to be standardized, and the acquisition rate of transgenic plants has been unsatisfactory. Furthermore, the germplasm resources of rubber trees are relatively narrow, and the introduction of wild species is also restricted, further reducing the available genetic diversity. Therefore, enhancing the efficiency of genetic transformation, optimizing the tissue culture process, and expanding the germplasm resource bank are all key issues that must be overcome in the future molecular breeding of rubber trees.

### **8.2 Need for integrative computational models for regulatory networks**

Latex biosynthesis and reflux reactions are jointly accomplished by the regulation of many levels, including genes, proteins, metabolites, and non-coding RNAs, etc. At present, although multi-omics data is constantly increasing, a single type of data often fails to fully explain its regulatory mechanism. Some studies have begun to attempt to combine GWAS, RNA-Seq, co-expression networks and enzyme networks to initially construct molecular networks related to rubber tree growth and latex yield. However, these analyses still lack a systematic and more predictive integrated model. In the future, it is necessary to establish systems biology methods based on multi-omics data to construct dynamic regulatory networks for latex synthesis and flow rate reactions, so as to more accurately identify key regulatory factors and verify their functions.

### **8.3 Prospects for sustainable latex production and climate resilience**

Global climate change and the continuous expansion of rubber planting areas into marginal environments have put greater pressure on the yield and adaptability of rubber trees. Studies have shown that the tolerance of rubber trees to adverse conditions such as drought and low temperature is related to certain specific genes and epigenetic regulation (such as DNA demethylation). Meanwhile, to enhance the sustainability of the rubber industry, attention should also be paid to the utilization of by-products. For instance, rubber seed oil can be used as biofuel and protein cake can be used as feed. All these contribute to promoting the transformation of the industry towards a circular economy. In the future, stress-resistant genes should be further explored. Combined with climate models and crop growth simulations, planting patterns and management measures should be optimized to achieve more efficient, sustainable and climate-resilient latex production.

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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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