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Biosynthesis of α-Linolenic Acid in *Eucommia ulmoides*: A Comprehensive Review

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Abstract α-Linolenic acid (ALA) is an essential ω-3 fatty acid that plays a crucial role in cardiovascular function, anti-inflammatory processes, and metabolic regulation. *Eucommia ulmoides*, a kand of Chinese tranditional medicinal plant with a very high ALA content, is the focus of this comprehensive review on ALA biosynthesis. The study explores the key biochemical pathways involved in ALA production, emphasizing the roles of dehydrogenases and elongases, as well as the gene regulatory mechanisms, including transcription factors and epigenetic modifications. The genetic basis of ALA biosynthesis is also analyzed, identifying key genes and molecular markers associated with ALA traits. Furthermore, the study reviews chromatographic and spectroscopic techniques used for ALA detection, along with modern molecular biological methods. Case studies on the genetic engineering of *Eucommia ulmoides* to enhance ALA yield provide practical insights for application. This study addresses the current challenges and future directions in ALA research, offering theoretical foundations and reference guidance for related studies in the fields of genetics and biochemistry.

Keywords α-linolenic acid; *Eucommia ulmoides*; Biosynthesis; Omega-3 fatty acids; Genetic engineering

1 Introduction

α-Linolenic Acid (ALA) is an essential omega-3 polyunsaturated fatty acid that plays a critical role in human health, particularly in cardiovascular function, anti-inflammatory responses, and neuroprotection. As the human body cannot synthesize ALA, it must be obtained through dietary sources, making plant-based ALA-rich foods of significant interest for nutritional and therapeutic purposes. ALA also serves as a precursor for the biosynthesis of other long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), further highlighting its importance in maintaining physiological balance and promoting overall health.

Medicinal plants have long been recognized as valuable sources of bioactive compounds, including ALA. Among these plants, *Eucommia ulmoides* stands out for its high ALA content and wide range of therapeutic properties. *Eucommia ulmoides*, commonly known as the Hardy Rubber Tree, has been traditionally used in Chinese medicine for its anti-hypertensive, anti-inflammatory, and bone-strengthening effects. Recent studies have shown that the plant's health benefits may be partially attributed to its rich ALA content, which underscores its significance in both traditional and modern medicine. Investigating the biosynthesis of ALA in *Eucommia ulmoides* can provide deeper insights into its medicinal potential and open new avenues for its application in functional foods, poultry feed and pharmaceuticals.

This paper provides a comprehensive analysis of ALA biosynthesis in *Eucommia ulmoides*, focusing on the key enzymes involved in ALA biosynthesis, the regulatory mechanisms of ALA biosynthesis, and a comparison of these pathways with those in other plant species. Through this study, the goal is to deepen the understanding of the molecular and biochemical processes underlying ALA biosynthesis in *Eucommia ulmoides*, offering scientific insights that can help optimize ALA production and enhance its utilization in medicinal applications.

2 Biochemical Pathways of α-Linolenic Acid Biosynthesis

2.1 Key enzymes involved in ALA biosynthesis

Desaturases are crucial enzymes in the biosynthesis of α-linolenic acid (ALA). These enzymes introduce double bonds into fatty acid chains, a key step in the formation of polyunsaturated fatty acids like ALA. In *Eucommia ulmoides*, the high expression of the ω-3 fatty acid desaturase coding gene EU0103017 has been identified as a significant factor contributing to the high ALA content in the plant. This enzyme catalyzes the conversion of linoleic acid (LA) to ALA by introducing a double bond at the ω-3 position, which is essential for the biosynthesis of ALA. Elongases are another group of enzymes that play a pivotal role in fatty acid metabolism by extending the carbon chain length of fatty acids. Although the specific elongases involved in ALA biosynthesis in *Eucommia ulmoides* have not been explicitly detailed, studies in mammalian systems have shown that elongases such as Elovl3, Elovl4, Elovl5, and Elovl6 are integral to the elongation process (Du et al., 2023). These enzymes add two-carbon units to the fatty acid chain, which is a critical step preceding the desaturation process that ultimately leads to the formation of ALA.

2.2 Gene regulation in the ALA biosynthetic pathway

Transcription factors are proteins that regulate the expression of genes involved in various biosynthetic pathways, including those for ALA. In *Eucommia ulmoides*, specific transcription factors that influence the expression of desaturase and elongase genes have not been fully characterized. However, the regulation of these enzymes is likely controlled by a complex network of transcription factors that respond to developmental and environmental cues, ensuring the proper synthesis of ALA. Epigenetic modifications, such as DNA methylation and histone modification, play a significant role in the regulation of gene expression. These modifications can influence the activity of genes involved in the ALA biosynthetic pathway by altering chromatin structure and accessibility. While specific epigenetic modifications in *Eucommia ulmoides* have not been extensively studied, it is plausible that such mechanisms are involved in the regulation of key enzymes like desaturases and elongases, thereby affecting ALA biosynthesis (Gregory et al., 2011).

2.3 Comparative analysis of ALA biosynthesis in *Eucommia ulmoides* and other plants

The biosynthetic pathway of ALA in *Eucommia ulmoides* shares several similarities with other plants. In general, the process involves the desaturation of linoleic acid to produce ALA, a reaction catalyzed by ω-3 fatty acid desaturases. This pathway is conserved across many plant species, indicating a common evolutionary origin for the enzymes involved in ALA biosynthesis. One unique aspect of ALA biosynthesis in *Eucommia ulmoides* is the exceptionally high expression of the ω-3 fatty acid desaturase coding gene EU0103017, which significantly contributes to the high ALA content in*Eucommia ulmoides* (Wang et al., 2022). This high expression level may be a result of specific regulatory mechanisms or evolutionary adaptations that enhance the plant's ability to produce ALA, distinguishing it from other species with lower ALA content. By understanding these biochemical pathways and regulatory mechanisms, researchers can better appreciate the unique aspects of ALA biosynthesis in *Eucommia ulmoides* and potentially apply this knowledge to improve ALA production in other plants (Lei et al., 2017).

3 Genetic Basis of α-Linolenic Acid Biosynthesis

3.1 Identification and characterization of ALA-related genes in *Eucommia ulmoides*

High-throughput sequencing has been instrumental in identifying genes involved in the biosynthesis of α-linolenic acid (ALA) in *Eucommia ulmoides*. Transcriptome sequencing of *E. ulmoides* seeds at different developmental stages has revealed numerous unigenes associated with fatty acid biosynthesis. For instance, among the 23 314 unigenes identified in the transcriptome of male and female flowers of *E. ulmoides* during the flowering period, 56 were related to fatty acid synthesis, including key enzymes such as 3-ketoacyl-ACP reductase and β-ketoacyl-ACP synthase II (Zhao et al. 2015). Another study utilized RNA-sequencing to identify genes related to glycolytic metabolism, which is crucial for providing intermediates for ALA biosynthesis (Feng et al., 2016). Functional annotation of the identified genes has provided insights into their roles in ALA biosynthesis. For example, genes encoding ω-3 fatty acid desaturase (FAD) enzymes, such as FAD2 and FAD3, have been characterized for their roles in converting linoleic acid to ALA. These genes were found to be highly expressed in tissues with high ALA content (Li et al., 2021). Additionally, the genome-wide analysis of the fatty acid desaturase gene family in *E. ulmoides* has identified key genes like EU0103017, which is highly expressed and significantly contributes to ALA biosynthesis.

3.2 Molecular markers associated with ALA biosynthesis

Single nucleotide polymorphism (SNP) markers have been identified and linked to ALA traits in *E. ulmoides*. These markers are crucial for understanding the genetic variation associated with ALA content and can be used in marker-assisted selection to enhance ALA levels in breeding programs. The identification of SNP markers in genes involved in the fatty acid biosynthesis pathway, such as those coding for desaturase enzymes, has been a significant advancement. Quantitative trait loci (QTL) mapping has been employed to identify genomic regions associated with ALA biosynthesis. This approach helps in pinpointing specific loci that contribute to the variation in ALA content among different *E. ulmoides* varieties. For instance, transcriptome analysis has facilitated the mapping of QTLs linked to the expression of key enzymes in the ALA biosynthetic pathway, providing a framework for future genetic studies (Xue et al., 2018).

3.3 Genetic modification strategies to enhance ALA content

CRISPR/Cas9 technology offers a powerful tool for the precise editing of genes involved in ALA biosynthesis. By targeting and modifying specific genes, such as those encoding FAD enzymes, it is possible to enhance the ALA content in *E. ulmoides*. This approach has the potential to create high-ALA varieties by knocking out negative regulators or enhancing the expression of positive regulators in the ALA biosynthetic pathway. Overexpression and gene silencing techniques have been employed to manipulate the expression of genes involved in ALA biosynthesis. For example, overexpressing genes like FAD3 in model organisms such as Arabidopsis thaliana has been shown to significantly increase ALA content (Figure 1) (Duan et al., 2021). Similarly, gene silencing techniques can be used to downregulate genes that negatively impact ALA accumulation, thereby enhancing the overall ALA content in *E. ulmoides* seeds. In conclusion, the genetic basis of α-linolenic acid biosynthesis in *Eucommia ulmoides* is being unraveled through high-throughput sequencing, functional annotation, and the identification of molecular markers. Genetic modification strategies, CRISPR/Cas9 and overexpression techniques, are expected to increase ALA content and successfully establish transgenic technology and CRISPR/Cas9 technology (Zhao et al., 2009; Wang et al., 2023), paving the way for the development of high-nutritional and medicinal value superior Eucommia species.

Duan et al. (2021) found that overexpression of the *PfFAD3.1* gene under the control of the CaMV35S promoter in Arabidopsis thaliana significantly altered both the expression levels of the transgene and the fatty acid (FA) composition in seeds. The transgenic plants exhibited marked increases in specific fatty acids, particularly C18:3, which is consistent with the role of *PfFAD3.1* in modulating FA desaturation pathways. Quantitative RT-PCR analysis revealed that the transgenic seeds had significantly higher *PfFAD3.1* expression compared to wild-type controls, confirming the successful integration and expression of the transgene. Additionally, fatty acid profiling demonstrated changes in both saturated and unsaturated FA content, indicating that *PfFAD3.1* plays a critical role in the biosynthesis and regulation of FAs in seeds. These findings highlight the potential for *PfFAD3.1* to enhance seed oil composition in plants through targeted genetic modification.

4 Role of Environmental Factors in ALA Biosynthesis

4.1 Influence of temperature on ALA production

Temperature plays a crucial role in the biosynthesis of α-linolenic acid (ALA) in various organisms, including *Eucommia ulmoides*. Studies have shown that low temperatures can significantly enhance the accumulation of ALA-rich lipids. For instance, in the microalga *Desmodesmus* sp., incubation at 5 ℃ resulted in a 1.5-fold increase in lipid content, with ALA constituting 44% of the total fatty acids (Sijil et al., 2019). This suggests that lower temperatures may create an optimal environment for ALA biosynthesis by possibly influencing the activity of enzymes involved in the fatty acid synthesis pathway. Temperature stress, particularly low-temperature stress, has been observed to affect ALA content in various species. In *Desmodesmus* sp., UV-treated cultures incubated at 5 °C showed a significant increase in lipid accumulation, with ALA fractions ranging from 39% to 42% of total fatty acids. This indicates that temperature stress, combined with other stress factors like UV treatment, can synergistically enhance ALA production. However, the specific mechanisms by which temperature stress influences ALA biosynthesis in *Eucommia ulmoides* require further investigation.

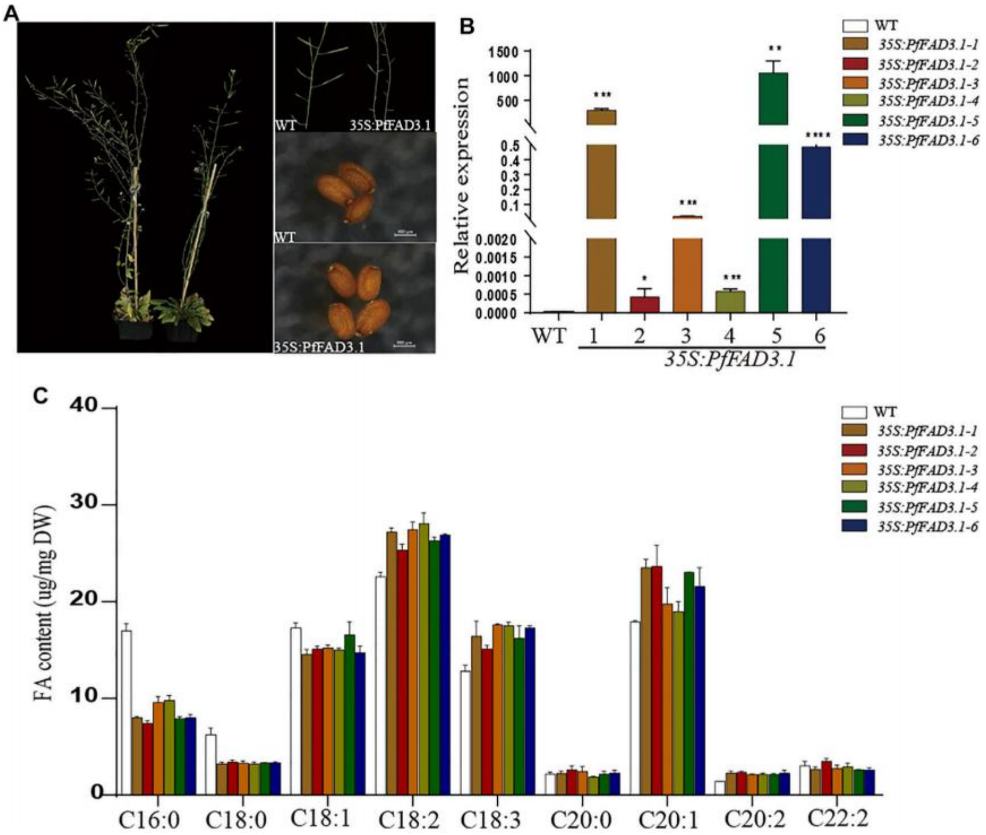

Figure 1 Effect of transgenic *PfFAD3.1* expression on FA composition in *A. thaliana* seeds (Adopted from Duan et al., 2021)

Image capton: (A) Transgenic CaMV35S::PfFAD3.1 Arabidopsis plants and seeds. (B) The expression of *PfFAD3.1* transgenic 35S::PfFAD3.1 Arabidopsis seeds determined by qRT-PCR. (C) FA composition in the seeds of transgenic 35S::PfFAD3.1 Arabidopsis. Error bars represent SD from at least three biological replicates. The asterisks indicate significant differences (*$p < 0.05$, Student's t-test) (Adopted from Duan et al., 2021)

4.2 Effect of soil nutrients and water availability

Soil nutrients, particularly nitrogen and phosphorus, are essential for the growth and metabolic activities of plants, including the biosynthesis of ALA. In *Desmodesmus* sp., nitrogen stress was found to increase the overall lipid content to 39% w/w, although the ALA fraction was reduced to 18% of total fatty acids (Zhao et al., 2019). This suggests that while nitrogen limitation can boost lipid accumulation, it may not necessarily favor ALA synthesis. The impact of phosphorus levels on ALA biosynthesis in *Eucommia ulmoides* remains to be elucidated, but it is likely that balanced nutrient availability is crucial for optimal ALA production. Water availability is another critical factor influencing ALA biosynthesis. Adequate water supply ensures proper physiological and metabolic functions, which are essential for the synthesis of fatty acids, including ALA. While specific studies on the correlation between water availability and ALA production in *Eucommia ulmoides* are limited, it is reasonable to infer that water stress could potentially affect ALA content by disrupting normal metabolic processes (Pan et al., 2012).

4.3 Impact of biotic stress on ALA biosynthesis

Biotic stress, such as pathogen attacks, can lead to significant changes in the metabolic profile of plants, including the biosynthesis of ALA (Wu et al., 2022). Pathogen-induced stress often triggers a defensive response in plants, which can alter the expression of genes involved in fatty acid metabolism. Although specific data on pathogen-induced changes in ALA content in *Eucommia ulmoides* are not available, it is plausible that such stress could either enhance or inhibit ALA biosynthesis depending on the nature and severity of the pathogen attack. Symbiotic relationships with microorganisms, such as mycorrhizal fungi and nitrogen-fixing bacteria, can influence the nutrient uptake and metabolic activities of plants, potentially affecting ALA biosynthesis. While there is no direct evidence linking symbiotic organisms to ALA production in *Eucommia ulmoides*, it is known that such relationships can enhance overall plant health and metabolic efficiency, which could indirectly support higher ALA synthesis. In summary, environmental factors such as temperature, soil nutrients, water availability, and biotic stress play significant roles in the biosynthesis of ALA in *Eucommia ulmoides*. Understanding these factors can help optimize conditions for enhanced ALA production, which is valuable for both nutritional and medicinal purposes. Further research is needed to elucidate the specific mechanisms by which these factors influence ALA biosynthesis in this species (Yuan et al., 2021).

5 Analytical Techniques for α-Linolenic Acid Detection

5.1 Chromatographic methods for ALA analysis

Gas chromatography (GC) is a widely used technique for the separation and analysis of fatty acids, including α-linolenic acid (ALA). One study demonstrated the use of a highly polar ionic phase column (SLB-IL111) to resolve minor geometrical isomers of ALA in linseed oil. The identification of these isomers was further confirmed using gas chromatography-electron ionisation mass spectrometry (GC-EIMS) and covalent adduct chemical ionisation tandem mass spectrometry (CACI-MS/MS) (Gómez-Cortés et al., 2016). This method highlights the capability of GC to distinguish between structurally similar polyunsaturated fatty acid isomers, making it a valuable tool for ALA analysis. Liquid chromatography (LC) is another essential technique for the separation of ALA and its isomers. A study utilized a cellulose tris (3,5-dichlorophenylcarbamate)-based chiral stationary phase to successfully separate ALA from its positional isomer, γ-linolenic acid (GLA). The optimized chromatographic conditions included a reversed-phase eluent system and UV detection, which allowed for the effective discrimination of these isomers. This method was further adapted for LC-MS/MS implementation, demonstrating its potential for quantifying ALA in various matrices (Ianni et al., 2020).

5.2 Spectroscopic techniques for ALA quantification

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique used for the structural elucidation and quantification of fatty acids, including ALA. NMR provides detailed information about the molecular structure, including the number and position of double bonds in fatty acids. This technique is particularly useful for confirming the identity of ALA and distinguishing it from other similar fatty acids. Fourier-transform infrared (FTIR) spectroscopy is another valuable tool for the analysis of fatty acids. FTIR can be used to identify functional groups and characterize the molecular structure of ALA. This technique is advantageous due to its rapid analysis time and minimal sample preparation requirements. FTIR spectroscopy can complement other analytical methods, providing additional confirmation of ALA's presence and structure (Yuan et al., 2021).

5.3 Advances in molecular techniques for ALA detection

Polymerase chain reaction (PCR)-based methods have been developed to detect and quantify genes involved in the biosynthesis of ALA. These techniques can amplify specific DNA sequences associated with ALA biosynthetic pathways, allowing for the identification of key genes and their expression levels. PCR-based methods are highly sensitive and specific, making them suitable for studying the genetic regulation of ALA production. Next-generation sequencing (NGS) has revolutionized the field of genomics, providing comprehensive insights into the genetic basis of ALA biosynthesis. A study on the chromosome-level genome of *Eucommia ulmoides* utilized PacBio and Hi-C technologies to assemble high-quality genomes, revealing key genes involved in ALA biosynthesis. The high expression of the ω-3 fatty acid desaturase coding gene (EU0103017) was identified as a significant factor contributing to the high ALA content in *E. ulmoides* (Figure 2) (Du et al., 2023). NGS enables the detailed analysis of entire genomes, facilitating the discovery of novel genes and regulatory mechanisms associated with ALA production.Through the whole-genome sequencing of

Eucommia ulmoides, we identified 9 genes encoding omega-3 fatty acid dehydrogenases (Zhao et al., unpublished data). By integrating these advanced analytical and molecular techniques, researchers can achieve a comprehensive understanding of ALA biosynthesis, detection, and quantification, ultimately contributing to the development of superior varieties and enhanced nutritional products.

6 Case Study

6.1 Case study of enhanced ALA production in *Eucommia ulmoides* through genetic engineering

Recent advancements in genetic engineering have significantly enhanced the production of α-linolenic acid (ALA) in *Eucommia ulmoides*. One notable study utilized transcriptome analysis to identify key genes involved in the biosynthesis of ALA. The high expression of the ω-3 fatty acid desaturase coding gene EU0103017 was found to be a crucial factor for the elevated ALA content in *E. ulmoides* (Figure 2) (Du et al., 2023). Additionally, research on transgenic models, such as Arabidopsis, has provided insights into overcoming the challenges associated with high ALA accumulation, such as embryonic developmental defects. Overexpression of lysophosphatidic acid acyltransferase 2 (LPAT2) was shown to rescue these defects by enhancing the esterification of ALA to triacylglycerol (TAG), thereby mitigating stress. These findings underscore the potential of genetic engineering in optimizing ALA production in *E. ulmoides*.

Figure 2 presents the reconstructed pathway of α-linolenic acid biosynthesis and metabolism in *Eucommia ulmoides*, with a heatmap showing the expression levels of key enzyme genes in the fruit, stem, leaf, and bark. The figure clearly indicates that several key enzyme genes responsible for α-linolenic acid biosynthesis exhibit significantly higher expression levels in the fruit and leaf compared to the stem and bark. Notably, the *EU0103017* gene, which encodes ω-3 fatty acid desaturase (FAD7), shows extremely high expression in the fruit and leaf, indicating its crucial role in α-linolenic acid biosynthesis. This high differential gene expression reveals the mechanism of α-linolenic acid accumulation in the fruit and leaf. In contrast, the lower expression of metabolism-related genes further supports the efficient accumulation of α-linolenic acid in these tissues.

6.2 Field trials and real-world applications of ALA-rich *Eucommia ulmoides*

Field trials have demonstrated the practical applications of ALA-rich *Eucommia ulmoides* in various industries. The comprehensive characterization of *E. ulmoides* seed oil revealed its high ALA content, making it a promising candidate for use in food, pharmaceuticals, and cosmetics. The oil's excellent physicochemical properties and high vitamin E content further enhance its value. Moreover, the successful engineering of staple oil crops like Brassica napus with increased ALA content using genes from Perilla frutescens highlights the feasibility of similar approaches in *E. ulmoides* (Yin et al., 2020). These real-world applications illustrate the potential economic and health benefits of cultivating ALA-rich *E. ulmoides*.

6.3 Lessons learned from case studies and future applications

The case studies on *Eucommia ulmoides* provide several valuable lessons for future applications. Firstly, the identification and manipulation of key genes involved in ALA biosynthesis are critical for enhancing ALA content. Secondly, addressing the challenges associated with high ALA accumulation, such as developmental defects, is essential for the successful application of genetic engineering techniques (Xue et al., 2023). Lastly, the real-world applications of ALA-rich *E. ulmoides* in various industries highlight the importance of comprehensive characterization and field trials to ensure the viability and economic potential of genetically engineered crops (Zhang et al., 2018). Future research should focus on optimizing genetic engineering strategies and expanding the applications of ALA-rich *E. ulmoides* to maximize its benefits. By integrating these insights, researchers and industry professionals can develop more effective strategies for enhancing ALA production in *Eucommia ulmoides* and other crops, ultimately contributing to improved health outcomes and economic growth.

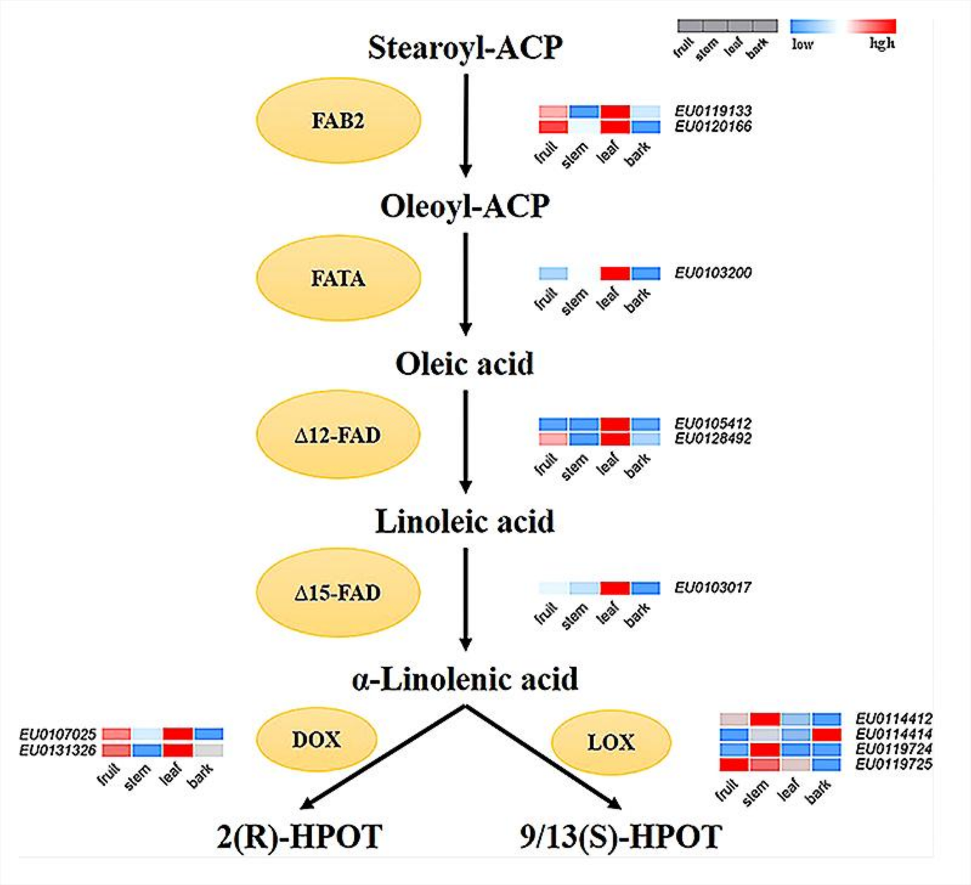

Figure 2 The reconstructed pathway of α-linolenic acid biosynthesis and metabolism in *Eucommia ulmoides* (Adopted from Du et al., 2023)

Image caption: FAB2, acyl-ACP desaturase; FATA, acyl-ACP thioesterase; D12-FAD, omega-6 fatty acid desaturase; D15-FAD, omega-3 fatty acid desaturase; LOX, lipoxygenase; DOX, alpha-dioxygenase. Various color blocks represent the normalized gene expression levels of candidate genes related to α-linolenic acid biosynthesis and metabolism in *Eucommia ulmoides*. The four boxes in one row of each heatmap (left to right) correspond to the expression levels in fruit, stem, leaf, and bark. Each row in the heatmap corresponds to one gene (Adopted from Du et al., 2023)

7 Health Benefits of α-Linolenic Acid from *Eucommia ulmoides*

7.1 Cardiovascular benefits of ALA

α-Linolenic acid (ALA) has been extensively studied for its cardiovascular benefits. Research indicates that increasing dietary ALA is associated with a significant reduction in the risk of total cardiovascular disease and fatal coronary heart disease. Meta-analyses of observational studies have shown that higher ALA intake can lead to a 10% lower risk of total cardiovascular disease and a 20% reduced risk of fatal coronary heart disease. Randomized controlled trials, such as the AlphaOmega trial, PREDIMED trial, and Lyon Diet Heart Study, have demonstrated that diets high in ALA can improve cardiovascular outcomes, although the AlphaOmega trial only showed a trend towards benefit. Additionally, ALA has been shown to reduce total cholesterol, LDL cholesterol, triglycerides, and blood pressure, which collectively contribute to its cardiovascular benefits. The anti-inflammatory properties of ALA also play a crucial role in its cardioprotective effects (Joris et al., 2019).

7.2 Anti-inflammatory effects of ALA

ALA exhibits significant anti-inflammatory properties, which are beneficial in various inflammatory conditions. Studies have shown that ALA can reduce the levels of inflammatory biomarkers such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) (Cambiaggi et al., 2023). In diabetic rats, ALA intake significantly reduced myocardial ischemia/reperfusion injury by decreasing inflammation and oxidative stress, highlighting its potential in managing inflammation-related cardiac events (Xie et al., 2011). The anti-inflammatory effects of ALA are partly mediated through its conversion to long-chain n-3 PUFAs and their oxylipin metabolites, which have immunomodulating effects. Furthermore, dietary ALA has been shown to suppress NF-κB activation and M1 macrophage polarization, leading to reduced adipose tissue inflammation and improved insulin sensitivity in high-fat diet-induced obesity models (Fan et al., 2019).

7.3 Potential role of ALA in metabolic disorders

ALA has shown promise in the management of metabolic disorders, including obesity, insulin resistance, and metabolic syndrome. Research indicates that ALA-enriched diets can attenuate high-fat diet-induced obesity and inflammation by promoting the bioconversion of long-chain n-3 PUFAs and their oxylipin metabolites. This bioconversion process is crucial for the metabolic benefits of ALA, as it leads to lipidomic modifications that improve insulin sensitivity and reduce hepatic triglyceride accumulation. Additionally, a meta-analysis reported a trend towards diabetes risk reduction with both dietary and biomarker ALA, although the evidence for ALA benefits in metabolic syndrome and obesity remains inconclusive (Sala-Vila et al., 2022). Further studies are needed to establish the clinical efficacy of ALA in managing metabolic disorders and to understand the underlying mechanisms better (Yuan et al., 2021). In summary, ALA from *Eucommia ulmoides* offers significant health benefits, particularly in cardiovascular health, anti-inflammatory effects, and potential roles in managing metabolic disorders. These benefits are supported by various studies and clinical trials, although further research is necessary to fully establish the clinical applications of ALA (Pan et al., 2012).

8 Traditional and Modern Applications of α-Linolenic Acid

8.1 Historical uses of *Eucommia ulmoides* for health

Eucommia ulmoides, commonly known as the hardy rubber tree, has been utilized in traditional Chinese medicine for centuries (Yuan et al., 2021). Historically, various parts of the plant, including its bark, leaves, and seeds, have been used to treat a range of ailments. The bark, in particular, is renowned for its properties in strengthening bones and muscles, alleviating hypertension, and promoting overall vitality. The seeds of *E. ulmoides* are rich in α-linolenic acid (ALA), a type of omega-3 fatty acid, which has been linked to numerous health benefits, including anti-inflammatory and cardiovascular protective effects (Sala-Vila et al., 2022).

8.2 Modern pharmaceutical applications of ALA

In contemporary medicine, α-linolenic acid (ALA) derived from *Eucommia ulmoides* has garnered significant attention for its potential therapeutic applications (Wang et al., 2011). Modern research has highlighted the high ALA content in *E. ulmoides* seeds, which constitutes up to 60% of the total fatty acids, making it a valuable source of this essential nutrient. ALA is known for its role in reducing the risk of chronic diseases such as heart disease, diabetes, and certain types of cancer. Additionally, the oil extracted from *E. ulmoides* seeds has been characterized for its excellent physicochemical properties, making it suitable for development as an edible oil with potential health benefits. The presence of vitamin E in the oil further enhances its antioxidant properties, contributing to its potential use in pharmaceuticals and nutraceuticals (Zhao et al., 2023).

8.3 Integration of traditional knowledge with modern research

The integration of traditional knowledge with modern scientific research has provided a comprehensive understanding of the health benefits of *Eucommia ulmoides* and its components. Traditional uses of *E. ulmoides* for strengthening the body and treating hypertension are supported by modern findings on the high ALA content and its associated health benefits. The advanced genomic studies on *E. ulmoides* have also shed light on the molecular mechanisms underlying ALA biosynthesis, paving the way for the development of superior varieties with enhanced health benefits (Zhu et al., 2020). By combining historical insights with contemporary research, there is a greater potential to develop new therapeutic applications and health products derived from *E. ulmoides*, thereby bridging the gap between traditional medicine and modern science (Li et al., 2020).

9 Challenges and Future Prospects in ALA Research

9.1 Current challenges in ALA biosynthesis research

The biosynthesis of α-linolenic acid (ALA) in *Eucommia ulmoides* faces several significant challenges. One of the primary issues is the incomplete understanding of the molecular mechanisms underlying ALA accumulation.

Although recent studies have identified key genes involved in glycolytic metabolism, which is crucial for providing the intermediates for ALA biosynthesis, the exact regulatory networks and pathways remain largely unexplored. Additionally, the complexity of the genome, including whole-genome duplication events, adds another layer of difficulty in pinpointing specific genes and their functions related to ALA biosynthesis. The variability in gene expression between different developmental stages and varieties of *E. ulmoides* further complicates the research, as it requires extensive and precise temporal and spatial gene expression analyses (Yu et al., 2020).

9.2 Potential breakthroughs in genetic and biochemical studies

Despite these challenges, there are promising avenues for breakthroughs in the genetic and biochemical studies of ALA biosynthesis. The availability of high-quality chromosome-level genomes for both male and female *E. ulmoides* provides a robust foundation for in-depth genetic studies. This genomic information can facilitate the identification of key regulatory genes and pathways involved in ALA biosynthesis. For instance, the high expression of the ω-3 fatty acid desaturase coding gene EU0103017 has been linked to high ALA content, suggesting a potential target for genetic manipulation2. Furthermore, advanced transcriptome sequencing technologies have enabled the identification and characterization of numerous unigenes related to glycolytic metabolism, which may play critical roles in ALA accumulation1. These technological advancements could lead to the development of superior *E. ulmoides* varieties with enhanced ALA content through targeted breeding and genetic engineering (Li, 2006).

9.3 Future directions for the application of ALA in medicine

The future application of ALA in medicine holds significant promise, particularly given its well-documented health benefits, including anti-inflammatory and cardioprotective effects. To fully harness the medicinal potential of ALA, future research should focus on optimizing the biosynthesis pathways in *E. ulmoides* to increase ALA yield. This could involve the use of CRISPR/Cas9 and other gene-editing technologies to enhance the expression of key biosynthetic genes identified in recent studies. Additionally, understanding the interaction between ALA biosynthesis and other metabolic pathways could lead to the development of *E. ulmoides* varieties with tailored fatty acid profiles for specific medical applications. Collaborative efforts between plant biologists, geneticists, and medical researchers will be essential to translate these findings into practical medical applications, potentially leading to new treatments and preventive strategies for various health conditions. By addressing these challenges and leveraging potential breakthroughs, the research on ALA biosynthesis in *Eucommia ulmoides* can pave the way for significant advancements in both plant science and medical applications.

10 Concluding Remarks

The biosynthesis of α-linolenic acid (ALA) in *Eucommia ulmoides* has been extensively studied, revealing several critical insights. The high-quality chromosome-level genome of *E. ulmoides* has provided valuable information on the genetic basis of ALA biosynthesis. Notably, the high expression of the ω-3 fatty acid desaturase coding gene (EU0103017) is a significant factor contributing to the high ALA content in *E. ulmoides*. Additionally, the characterization of *E. ulmoides* seed oil has shown that it contains approximately 61.36% linolenic acid, making it a promising candidate for various applications in food, pharmaceuticals, and cosmetics. Furthermore, the identification and expression analysis of glycolytic pathway genes in developing kernels of *E. ulmoides* have highlighted the role of glycolytic metabolism in ALA accumulation, with several genes showing higher expression in high-ALA varieties.

The findings from these studies open several avenues for future research. Firstly, the detailed genomic information can be leveraged to explore the regulatory mechanisms governing ALA biosynthesis and sex differentiation in *E. ulmoides*. This could lead to the development of superior varieties with enhanced ALA content and other desirable traits. Secondly, further research into the physicochemical properties and potential applications of *E. ulmoides* seed oil could facilitate its commercialization and utilization in various industries. Lastly, the role of glycolytic pathway genes in ALA accumulation warrants deeper investigation, particularly in understanding how these genes can be manipulated to increase ALA content in *E. ulmoides*kernels.

The high content of α-linolenic acid in *Eucommia ulmoides* underscores its significance as a valuable source of this essential fatty acid. ALA is known for its numerous health benefits, including anti-inflammatory properties and cardiovascular protection. The comprehensive understanding of ALA biosynthesis in *E. ulmoides* not only enhances our knowledge of plant lipid metabolism but also paves the way for the development of functional foods and nutraceuticals derived from this plant. The continued research and development in this area hold great promise for improving human health and well-being through the utilization of *E. ulmoides* and its derivatives.

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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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