Genomics and Applied Biology 2024, Vol.15, No.3, 142-152
http://bioscipublisher.com/index.php/gab

Research Insight Open Access

Integrating Functional Genomics with Breeding in *Eucommia ulmoides*

Xiaoyan Ran [1], Ying Zhu [2], Degang Zhao [1,2]

1 The Key Laboratory of Plant Resources Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), College of Life Sciences/Institute of Agro-Bioengineering, Guizhou University, Guiyang, 550025, China

2 Plant Conservation & Breeding Technology Center, Guizhou Key Laboratory of Agricultural Biotechnology/Biotechnology Institute of Guizhou Province, Guizhou Academy of Agricultural Sciences, Guiyang, 550006, China

Corresponding author: dgzhao@gzu.edu.cn

Genomics and Applied Biology, 2024, Vol.15, No.3 doi: 10.5376/gab.2024.15.0016

Received: 02 Apr., 2024

Accepted: 11 May, 2024

Published: 27 May, 2024

Preferred citation for this article:

Ran X.Y., Zhu Y., and Zhao D.G., 2024, Integrating functional genomics with breeding in *Eucommia ulmoides*, Genomics and Applied Biology, 15(3): 142-152 (doi: 10.5376/gab.2024.15.0016)

Abstract This involved constructing high-density genetic maps, analyzing quantitative trait loci (QTL) for growth traits, and identifying key genes involved in various biological processes. The study successfully constructed a high-density genetic map using single-nucleotide polymorphism (SNP) markers, covering 90% of the *E. ulmoides* genome with a total genetic distance of 4051.11 cM and an average marker distance of 0.45 cM. A total of 44 QTLs associated with growth traits were identified, along with 33 candidate genes related to energy storage, signal transmission, hormones, and metabolic pathways. Additionally, the genome of *E. ulmoides* was sequenced, revealing insights into sex differentiation and α-linolenic acid biosynthesis. The study also identified 71 NAC transcription factors and their potential role in rubber biosynthesis, and 119 MYB transcription factors involved in growth and development. The integration of functional genomics with breeding in *Eucommia ulmoides* has provided a solid foundation for future genetic improvement and breeding programs. The identification of key QTLs and candidate genes will facilitate targeted breeding strategies to enhance desirable traits, thereby improving the economic and ecological value of this important species.

Keywords *Eucommia ulmoides*; Functional genomics; Breeding; SNP markers; QTL analysis; Genetic map; NAC transcription factors; MYB transcription factors; α-linolenic acid; Rubber biosynthesis

1 Introduction

Eucommia ulmoides, commonly known as the hardy rubber tree, is a unique species renowned for its dual utility in both traditional medicine and industrial applications. The tree is the sole member of the family Eucommiaceae and is highly valued for its ability to produce rubber and various medicinal compounds. The rubber produced by *E. ulmoides* is distinct due to its reliance on the methylerythritol-phosphate (MEP) pathway, which is different from the mevalonate (MVA) pathway used by other rubber-producing species like *Hevea brasiliensis*. Additionally, the tree's leaves and bark are rich in chlorogenic acid, a compound with significant medicinal properties (Li et al., 2020).

Despite its economic and medicinal importance, the cultivation and breeding of *E. ulmoides* face several challenges. One of the primary issues is the dioecious nature of the species, which means that male and female flowers are produced on separate trees. This characteristic complicates early sex identification, making traditional breeding methods inefficient and time-consuming (Wang et al., 2020). Furthermore, the genetic regulation of sex determination in *E. ulmoides* is complex, involving various MADS-box transcription factors that exhibit sex-specific expression patterns (Zhang et al., 2023b). These challenges hinder the optimization of breeding programs aimed at improving yield and quality.

Integrating functional genomics into breeding programs offers a viable solution to the challenges faced in *Eucommia ulmoides* cultivation. High-quality genome assemblies and advanced genomic tools provide deep insights into the genetic and molecular mechanisms of important traits such as rubber biosynthesis and sex determination (Li et al., 2020; Zhang et al., 2023b). For instance, the recently acquired high-quality haploid genome assembly of *Eucommia ulmoides* has greatly enhanced our understanding of its genomic structure and

evolution, providing direction for targeted genetic engineering (Li et al., 2020). Additionally, the identification of sex-related molecular markers through technologies like ddRAD-seq can facilitate early and accurate sex determination, simplifying the breeding process (Wang et al., 2020).

This study aims to integrate functional genomics with traditional breeding methods to overcome the existing challenges in *E. ulmoides* cultivation. By leveraging high-quality genomic data and advanced molecular techniques, we seek to enhance the efficiency and effectiveness of breeding programs. The specific objectives of this study include elucidating the genetic basis of key traits such as rubber biosynthesis and sex determination, developing reliable molecular markers for early sex identification, and applying genomic insights to improve the industrial and medicinal value of *E. ulmoides* through targeted breeding and genetic engineering strategies. By addressing these objectives, this study will contribute to the sustainable cultivation and commercial exploitation of *E. ulmoides*, ensuring its continued significance in both traditional medicine and industry.

2 Functional Genomics: A Tool for Breeding

2.1 Definition and key components of functional genomics

Functional genomics is an interdisciplinary field that aims to understand the complex relationships between genetic information and phenotypic traits. It involves the study of gene expression, protein function, and regulatory networks to elucidate the roles of genes and their interactions within an organism. Key components include:

Gene Expression: Investigating how genes are transcribed into RNA and translated into proteins, and how these processes are regulated.

Protein Function: Understanding the roles of proteins encoded by genes, including their interactions and pathways.

Regulatory Networks: Mapping the interactions between genes, proteins, and other molecules to understand the regulatory mechanisms controlling gene expression and function (Ge et al., 2003; Kumar et al., 2020).

2.2 Role of functional genomics in understanding key traits

Functional genomics plays a crucial role in understanding key traits in *Eucommia ulmoides*, such as latex production and stress resistance. By analyzing gene expression and protein function, researchers can identify the genetic basis of these traits and how they are regulated. For instance, the identification of candidate genes related to energy storage, signal transmission, hormones, and metabolic pathways can provide insights into growth traits and other important characteristics (Jin et al., 2020; Liu et al., 2022). Additionally, understanding the regulatory networks involved in stress responses can help in developing more resilient varieties (Varshney et al., 2005; Weckwerth et al., 2020).

2.3 Advances in genomic sequencing technologies applied to *E. ulmoides*

In recent years, breakthroughs in genome sequencing technology have significantly enhanced our ability to study *Eucommia ulmoides* at the molecular level. High-quality genome assemblies have been achieved using technologies such as PacBio and Hi-C, providing detailed insights into the genetic makeup of this species. These advancements have made it possible to construct high-density genetic maps and identify quantitative trait loci (QTL) associated with important traits (Li et al., 2020; Liu et al., 2022; Du et al., 2023). The availability of comprehensive genomic data has facilitated the application of functional genomics in breeding programs, making the selection of desirable traits more precise and efficient (Poland, 2015; Kumar et al., 2020).

2.4 Case study: genomic studies on latex production in *E. ulmoides*

A notable case study in the application of functional genomics to *E. ulmoides* is the investigation of latex production. Researchers have utilized high-quality genome assemblies to identify the pathways involved in rubber biosynthesis. It was found that *E. ulmoides* relies predominantly on the methylerythritol-phosphate (MEP) pathway for isoprenyl diphosphate synthesis, which is crucial for rubber production. This pathway operates

mainly in the trans-polyisoprene-containing leaves and central peels of the plant (Li et al., 2020) (Figure 1). Such insights are invaluable for breeding programs aimed at enhancing latex yield and quality, demonstrating the practical applications of functional genomics in improving economically important traits (Li et al., 2020; Du et al., 2023).

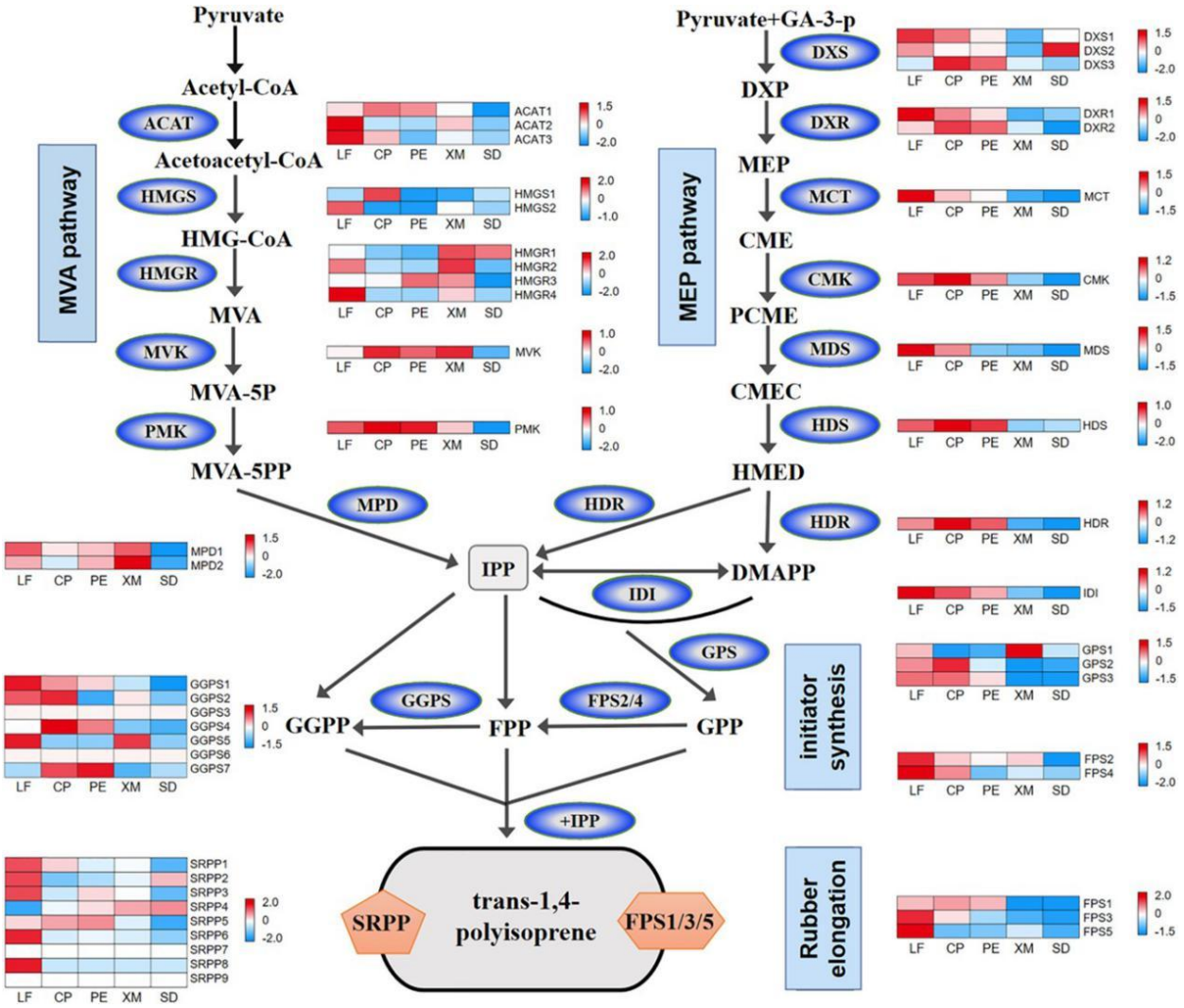

Figure 1 The *E. ulmoides* rubber biosynthesis pathway and expression profiles of genes involved in the pathway (Adopted from Li et al., 2020)

Image caption: The expression level is presented by log2-transformed fragments mapped per kilobase of transcript length per million total mapped reads (log2-FPKM). ACAT acetyl-coenzyme A (CoA) C-acetyltransferase; HMGS hydroxymethylglutaryl-CoA synthase; HMGR hydroxymethylglutaryl-CoA reductase; MVK mevalonate kinase; PMK 5-phosphomevalonate kinase; MPD mevalonate pyrophosphate decarboxylase; DXS 1-deoxy-d-xylulose 5-phosphate synthase; DXR 1-deoxy-d-xylulose 5-phosphate reductoisomerase; MCT 2-C-methyl-d-erythritol 4-phosphate cytidylyltransferase; CMK 4-(cytidine 5′-diphospho)-2-C-methyl-d-erythritol kinase; MDS 2-C-methyl-d-erythritol 2,4-cyclodiphosphate synthase; HDS 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HDR 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; IDI isopentenyl diphosphate isomerase; GPS geranyl diphosphate synthase; FPS farnesyl diphosphate synthase; GGPS, geranylgeranyl diphosphate synthase; SRPP small rubber particle protein; Acetyl-CoA acetyl coenzyme-A; Acetoacetyl-CoA, 3-acetoacetyl-CoA; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MVA mevalonate; MVA-5P mevalonate-5-phosphate; MVA-5PP mevalonate-5-diphosphate; GA-3-P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-d-xylulose 5-phosphate; MEP 2-C-methyl-d-erythritol 4-phosphate; CME 4-(cytidine 5′-diphospho)-2-C-methyl-d-erythritol; PCME 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-d-erythritol; CMEC 2-C-methyl-d-erythritol 2,4-cyclodiphosphate; HMED 4-hydroxy-3-methylbut-2-enyl diphosphate; IPP isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP geranyl diphosphate; FPP farnesyl diphosphate; GGPP, geranylgeranyl diphosphate. LF leaf; CP central peel; PE peel edge; XM xylem; SD seed (Adopted from Li et al., 2020)

3 Breeding Strategies in *E. ulmoides*

3.1 Traditional breeding approaches: limitations and successes

The traditional breeding methods for *Eucommia ulmoides* mainly rely on phenotypic selection and hybrid breeding. These methods have been successful in improving certain traits, such as growth rate and yield. However, they also have significant limitations. *Eucommia ulmoides* takes seven to eight years to flower from the seedling stage, and it is difficult to distinguish the sex before flowering using morphological or cytological methods, making early identification of superior traits challenging and extending the breeding cycle (Wang et al., 2011). Furthermore, the genetic basis of many important traits remains poorly understood, limiting the effectiveness of traditional selection methods (Li et al., 2014; Jin et al., 2020). Despite these challenges, traditional breeding has laid the foundation for more advanced technologies by providing genetic diversity and heritability of traits, giving us a fundamental understanding of the breeding process.

3.2 Modern breeding techniques: marker-assisted selection, genomic selection

Modern breeding techniques have revolutionized traditional Eucommia breeding by integrating molecular markers and genomic data. Marker-assisted selection (MAS) utilizes the close linkage between molecular markers and genes determining target traits. By detecting molecular markers, the presence of the desired gene can be identified, allowing for the selection of target traits. MAS offers advantages such as speed, accuracy, and being unaffected by environmental conditions, making it particularly effective in identifying and selecting quantitative trait loci (QTL) with large effects (Li et al., 2014; Jin et al., 2020). For example, high-density genetic maps developed using single nucleotide polymorphism (SNP) markers have facilitated the identification of QTLs related to growth traits, enabling more precise selection (Liu et al., 2022).

Genomic selection (GS) is a new method for breeding selection that uses high-density markers covering the entire genome. It goes a step further than marker-assisted selection by using genome-wide markers to predict an individual's breeding value. This method can capture the effects of all QTLs, including small-effect QTLs, thereby improving selection accuracy (Goddard and Hayes, 2007; Heslot et al., 2015; Merrick et al., 2022). GS has shown outstanding performance in accelerating breeding cycles and increasing genetic gain per unit of time, making it a valuable tool for improving complex traits in *Eucommia ulmoides* (Varshney et al., 2017).

3.3 Hybrid breeding strategies and their application in *E. ulmoides*

Hybrid breeding strategies combine traditional and modern technologies to maximize genetic gain while controlling inbreeding. One such approach is Mate Selection (MS), which uses optimization algorithms to select the best individuals and their pairings. This method has been shown to reduce inbreeding and increase genetic gain in other species, and it also holds potential for *Eucommia ulmoides* (Tchounke et al., 2022).

The integration of functional markers (FMs) into hybrid breeding strategies can further enhance the precision of selection. FMs are closely associated with phenotypic traits and can be used to directly select for desirable genes, thereby increasing selection efficiency (Salgotra and Stewart, 2020). The use of FMs in combination with MAS and GS can provide a comprehensive approach to breeding *E. ulmoides*, addressing both the limitations of traditional methods and the complexities of modern genomic techniques.

4 Integrating Functional Genomics into Breeding Programs

4.1 How functional genomics enhances trait selection and breeding efficiency

Functional genomics plays a key role in improving trait selection and breeding efficiency by providing detailed insights into the genetic basis of target traits. By identifying and characterizing functional genes, breeders can more efficiently enhance traits such as yield, disease resistance, and stress tolerance through marker-assisted selection (MAS) and genomic selection (GS). For example, the use of functional markers (FMs) allows direct selection of genes associated with phenotypic traits, thus improving selection efficiency and accelerating the breeding of superior varieties. The precision of breeding has greatly improved. Furthermore, advancements in high-throughput sequencing and genome editing have made variety development faster and more precise, further enhancing breeding efficiency (Salgotra and Stewart, 2020).

4.2 Identification of key genes responsible for desirable traits

Identifying key genes responsible for target traits such as growth, disease resistance, and drought tolerance is the foundation of functional genomics. In *Eucommia ulmoides*, several studies have focused on quantitative trait loci (QTL) analysis and identified candidate genes related to growth traits. For instance, one study identified 89 putative QTLs associated with growth traits, including 25 related to tree height, 32 related to ground diameter, and 15 related to crown diameter (Jin et al., 2020). Another study used single nucleotide polymorphism (SNP) markers to construct a high-density genetic map, identifying 44 QTLs related to growth traits and 33 candidate genes involved in energy storage, signal transduction, hormones, and metabolic pathways (Liu et al., 2022). These findings provide a solid foundation for improving the genetic basis of *Eucommia ulmoides* through genome-assisted breeding.

4.3 Case study: genetic improvement of stress resistance through genomics-assisted breeding

A notable case study in the genetic improvement of stress resistance through genomics-assisted breeding can be seen in the application of genomic selection (GS) in various crops and tree species. GS uses genetic markers covering the whole genome to predict breeding values with high accuracy, thereby facilitating the rapid selection of superior genotypes (Goddard and Hayes, 2007; Crossa et al., 2017). In *Eucommia ulmoides*, the integration of functional genomics with breeding programs has led to the identification of key genes and QTLs associated with stress resistance traits. For instance, the MYB transcription factor family in *E. ulmoides* has been studied for its role in regulating rubber biosynthesis and stress responses, providing valuable insights for breeding programs aimed at enhancing stress resistance (Hu et al., 2023) (Figure 2). By leveraging these genomic tools and insights, breeders can develop *E. ulmoides* varieties with improved stress resistance, contributing to the sustainability and productivity of this economically important tree species.

5 Genomic Tools and Resources for *E. ulmoides*

5.1 Overview of current genomic databases and resources for *E. ulmoides*

The genomic resources for *Eucommia ulmoides* have significantly expanded in recent years, providing valuable data for both basic and applied research. A high-quality chromosome-level genome assembly for the female *E. ulmoides* was obtained using PacBio and Hi-C technologies, resulting in a 1.01 Gb genome with 17 pseudochromosomes and 31 665 protein-coding genes. This assembly also facilitated the reassembly of the male genome, enhancing its scaffold N50 to 48.30 Mb and increasing the number of predicted genes by 11 266 (Du et al., 2023). Additionally, a high-quality haploid genome assembly was generated, improving the scaffold N50 to 53.15 Mb and providing insights into rubber biosynthesis and genome evolution (Li et al., 2020). These genomic assemblies are crucial for understanding the genetic basis of important traits and for advancing breeding programs.

5.2 High-throughput sequencing platforms and bioinformatics tools for functional analysis

High-throughput sequencing platforms such as Illumina, PacBio, and Hi-C technologies have played a crucial role in generating comprehensive genomic and transcriptomic data for *Eucommia ulmoides*. For example, the Illumina platform was used for sequencing the transcriptomes of male and female flower buds, identifying 75 065 unigenes and a large number of single nucleotide polymorphisms (SNPs) (Liu et al., 2016). PacBio and Hi-C technologies were employed to produce high-quality genome assemblies, which are essential for detailed functional genomics studies (Li et al., 2020; Du et al., 2023). Bioinformatics tools such as Trinity have been used for de novo assembly of large datasets, and various annotation pipelines have been analyzed to provide in-depth insights into gene function and regulation for functional analysis of *Eucommia ulmoides* (Liu et al., 2016).

5.3 Importance of genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping

Genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping are pivotal for identifying genetic variants associated with important traits in *E. ulmoides*. A high-density genetic map constructed using genotyping-by-sequencing (GBS) identified 191 095 SNPs and mapped 10 103 SNP markers across 17 linkage groups, covering 90% of the genome. This map facilitated the detection of 44 QTLs associated with growth traits, providing a foundation for marker-assisted selection (Liu et al., 2022). Another study updated the genetic linkage

map and identified 89 QTLs related to growth traits over a decade, highlighting the potential for long-term genetic improvement (Jin et al., 2020). These approaches are essential for dissecting the genetic architecture of complex traits and enhancing breeding efficiency in *E. ulmoides*.

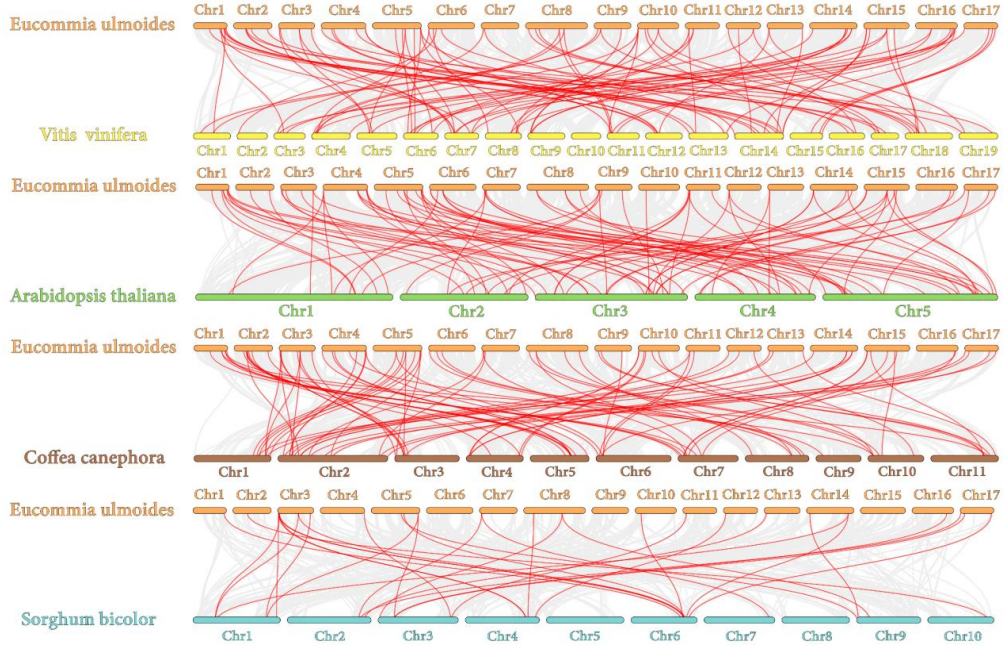

Figure 2 Collinearity analyses of the MYB gene family between *E. ulmoides* and four other species (Adopted from Hu et al., 2023)
Image caption: From top to bottom, the species collinearity analysis of *E. ulmoides*-Vitis vinifera (yellow), *E. ulmoides*-Arabidopsis thaliana (green), *E. ulmoides*-Coffea canephora (brown), *E. ulmoides*-Sorghum bicolor (blue). Gray lines in the background indicate the collinear blocks within *E. ulmoides* and different plant genomes, whereas red lines highlight syntenic MYB gene pairs (Adopted from Hu et al., 2023)

6 Challenges and Limitations in Integrating Functional Genomics and Breeding

6.1 Technical challenges: data analysis, genomic data integration, and interpretation

Integrating functional genomics with breeding in *Eucommia ulmoides* presents several technical challenges. One significant issue is the complexity of data analysis and the integration of vast genomic datasets. For instance, the high-quality chromosome-level genome assembly of *E. ulmoides*, which includes 31 665 protein-coding genes, requires sophisticated bioinformatics tools for accurate data interpretation and integration (Du et al., 2023). Additionally, the construction of high-density genetic maps using genotyping-by-sequencing (GBS) and single-nucleotide polymorphism (SNP) markers involves managing and analyzing large volumes of data, which can be technically demanding (Liu et al., 2022). The identification and mapping of quantitative trait loci (QTL) for growth traits further complicate the data analysis process, as it requires long-term phenotypic data collection and advanced statistical methods to correlate genetic markers with phenotypic traits (Li et al., 2014; Jin et al., 2020).

6.2 Economic and logistical challenges in deploying advanced genomics in breeding programs

Deploying advanced genomic technologies in breeding programs for *E. ulmoides* also faces economic and logistical challenges. The cost of high-throughput sequencing technologies, such as PacBio and Hi-C, and the subsequent data analysis can be prohibitive for many research institutions and breeding programs (Li et al., 2020; Du et al., 2023). Additionally, the establishment and optimization of techniques like AFLP (Amplified Fragment Length Polymorphism) require significant investment in laboratory infrastructure and technical expertise (Dawei et al., 2010; Wang et al., 2011). Logistically, the long juvenile phase of *E. ulmoides*, which delays the identification of sex and other important traits, poses a challenge for timely breeding interventions (Wang et al., 2011). The need for extensive field trials to validate genomic predictions further adds to the logistical burden, requiring substantial time and resources (Jin et al., 2020; Liu et al., 2022).

6.3 Solutions and future directions: multi-omics integration, collaborative research efforts

To overcome these challenges, several solutions and future directions can be considered. One promising approach is to integrate multi-omics data, including genomics, transcriptomics, and metabolomics, to gain a comprehensive understanding of the genetic and molecular mechanisms of key traits in *Eucommia ulmoides* (Li et al., 2020; Du et al., 2023; Hu et al., 2023). Collaborative research at both national and international levels can help pool resources and expertise, making advanced genomic technologies more accessible and cost-effective (Wang et al., 2018; Zhang et al., 2023a). Additionally, the development of molecular markers such as sex-specific AFLP and SCAR markers can facilitate early identification of important traits, thereby accelerating breeding cycles (Wang et al., 2011). Investments in bioinformatics infrastructure and training can also effectively enhance data analysis and interpretation capabilities, ensuring that the large volumes of genomic data generated are effectively utilized in breeding programs (Jin et al., 2020; Liu et al., 2022; Du et al., 2023).

7 Future Prospects of Functional Genomics in *E. ulmoides* Breeding

7.1 Potential breakthroughs in crop improvement

Integrating functional genomics into the breeding of *Eucommia ulmoides* holds great promise for significant breakthroughs in crop improvement. Advances in genome editing technologies, especially CRISPR/Cas9, have revolutionized this field by offering an efficient and flexible method for precise gene editing, making it possible to modify plant genomes with accuracy. This technology allows for the deletion of detrimental traits and the addition of beneficial characteristics, thereby accelerating the development of novel plant varieties with improved traits such as disease resistance, drought tolerance, and enhanced nutritional profiles (Bortesi and Fischer, 2015; Arora and Narula, 2017; Rao and Wang, 2021). Additionally, the ability to generate diverse cis-regulatory alleles through CRISPR/Cas9-mediated editing of promoters can provide beneficial quantitative variation for breeding, which is crucial for improving complex traits like yield and plant architecture (Rodríguez-Leal et al., 2017).

7.2 Exploring the role of CRISPR-Cas9 and other gene-editing technologies in accelerating breeding

CRISPR-Cas9 and its variants have emerged as powerful tools for accelerating the breeding process in *E. ulmoides*. The technology's ability to introduce site-specific double-stranded DNA breaks and facilitate precise genome modifications has made it a cornerstone of modern plant breeding (Bortesi and Fischer, 2015; Zhang et al., 2017). Base editing, a derivative of CRISPR/Cas9, allows for precise single-nucleotide changes without the need for double-strand breaks, offering a new avenue for creating desirable traits in crops (Li et al., 2023). The development of delivery systems, such as DNA-free methods and CRISPR ribonucleoproteins (RNPs), has further enhanced the efficiency and specificity of genome editing, making it a more viable option for practical breeding applications (Arora and Narula, 2017; Chen et al., 2019) (Figure 3). These advancements are expected to significantly reduce the time required to develop new *E. ulmoides* varieties with improved traits.

7.3 Collaborative efforts between academia and industry in genomics-based breeding initiatives

The successful integration of functional genomics into *E. ulmoides* breeding will require robust collaboration between academic institutions and industry stakeholders. Academia can contribute cutting-edge research and technological innovations, while industry can provide the resources and infrastructure necessary for large-scale breeding programs. Collaborative efforts have already shown promise in other crops, where partnerships have led to the development of genome-edited varieties with enhanced traits (Wang et al., 2019; Ahmad et al., 2020). By leveraging the strengths of both sectors, it is possible to accelerate the translation of genomic research into practical breeding solutions. Such collaborations can also facilitate the sharing of genomic data, the development of standardized protocols, and the establishment of regulatory frameworks to ensure the safe and effective use of genome editing technologies in crop improvement (Wan et al., 2021).

8 Concluding Remarks

Functional genomics holds great potential for revolutionizing *Eucommia ulmoides* breeding. The availability of high-quality male and female plant genome assemblies provides a solid foundation for understanding the genetic basis of key traits, including sex differentiation and α-linolenic acid biosynthesis. The identification of sex-specific markers, such as SCAR markers for early sex identification, can significantly improve breeding

efficiency by selecting desired traits at an early stage. Moreover, the construction of high-density genetic maps and the identification of quantitative trait loci (QTL) associated with growth traits offer valuable insights into the genetic and molecular mechanisms underlying these traits, promoting marker-assisted selection and genetic improvement. The integration of transcriptome analysis further identifies differentially expressed genes and potential sex-related genes, offering deeper insights into the genetic regulation of sexual dimorphism and other important traits. Overall, functional genomics provides a comprehensive toolkit for accelerating the breeding of high-quality *Eucommia ulmoides* varieties, enhancing their potential in medicinal, economic, and ecological value.

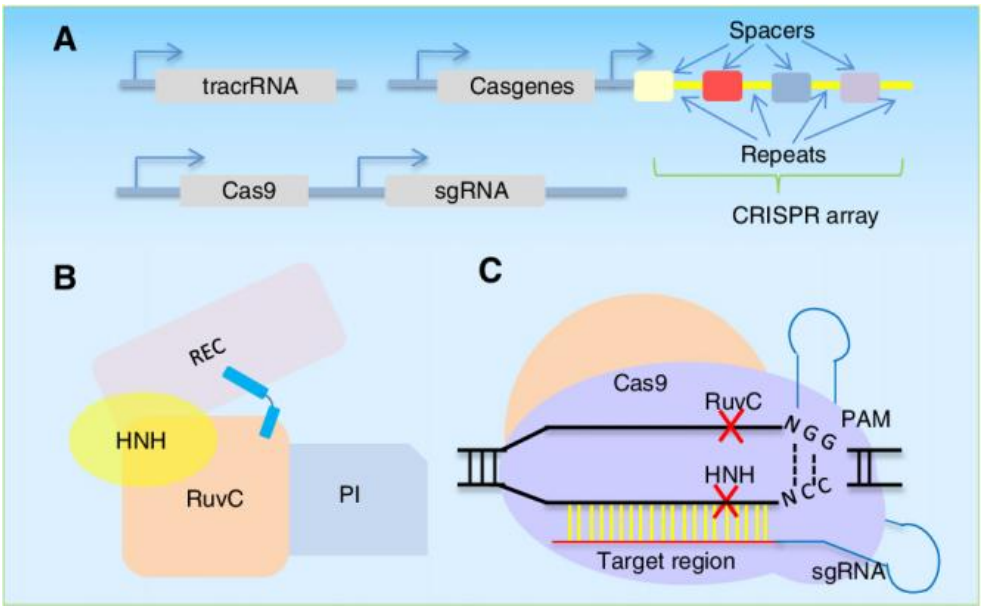

Figure 3 CRISPR-Cas9 gene editing technology (to make appropriate modifications from Arora and Narula, 2017)

Image caption: A. The double-strand break (DSB) is repaired by a non-homologous end join (NHEJ) repair mechanism, introducing insertion or deletion (indels) mutations[Knockcut]. B. The Homology-direct repair (HDR) mechanism is used to Repair of double-strand break (DSB) and introduce specific mutations or foreign sequences on target genes [Knockin] (to make appropriate modifications from Arora and Narula, 2017)

Moving forward, several key priorities should be addressed to fully harness the potential of functional genomics in *E. ulmoides* breeding:

Enhanced Genomic Resources: Continued efforts to improve the quality and completeness of genomic assemblies, including the integration of additional omics data such as proteomics and metabolomics, will provide a more comprehensive understanding of the genetic architecture of *E. ulmoides*.

Functional Characterization of Key Genes: Functional validation of candidate genes identified through QTL mapping and transcriptome analyses is crucial. Techniques such as CRISPR/Cas9-mediated gene editing can be employed to elucidate the roles of these genes in trait expression and regulation.

Development of Molecular Markers: The identification and validation of additional molecular markers, including SNPs and SSRs, will enhance the precision and efficiency of marker-assisted selection in breeding programs.

Breeding for Specific Traits: Focused breeding efforts should target specific traits of economic and ecological importance, such as rubber biosynthesis, growth traits, and stress resistance. The integration of functional genomics with traditional breeding methods can accelerate the development of high-yielding and resilient *E. ulmoides*varieties.

Conservation and Genetic Diversity: Ensuring the conservation of genetic diversity within *E. ulmoides* populations is essential for sustainable breeding. Studies on genetic diversity and population structure should inform breeding strategies to maintain a broad genetic base.

Collaborative Research and Data Sharing: Collaborative efforts among researchers, breeders, and industry stakeholders will be vital for the successful application of functional genomics in *E. ulmoides* breeding. Establishing databases and platforms for data sharing and collaboration can facilitate the exchange of knowledge and resources.

Acknowledgments

We are grateful to the reviewers for their valuable comments and suggestions on the manuscript of this study. These constructive feedbacks were crucial in refining the thesis and improving the quality of the research.

Funding

This research was funded by a grant from the National Natural Science Foundation of China [31870285, 30660146], National High Tech nology Research and Development Program of China ("863" Program) [grant number 2013AA102605-05], National Major Project of Cultivating New Varieties of Genetically Modified Organisms [grant no. 2016ZX08010003- 009], Guizhou Province High-Level Innovative Talent Training Program Project ([2016]4003), Guizhou Academy of Agricultural Sciences Talent Special Project (No. 2023-02 and No. 2024-02).

Conflict of Interest Disclosure

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

References

Ahmad S., Wei X., Sheng Z., Hu P., and Tang S., 2020, CRISPR/Cas9 for development of disease resistance in plants: recent progress, limitations and future prospects, Briefings in functional genomics. 19(1): 26-39.
https://doi.org/10.1093/bfgp/elz041

Arora L., and Narula A., 2017, Gene editing and crop improvement using CRISPR-Cas9 system, Frontiers in Plant Science, 8:1932.
https://doi.org/10.3389/fpls.2017.01932

Bortesi L., and Fischer R., 2015, The CRISPR/Cas9 system for plant genome editing and beyond, Biotechnology Advances, 33(1):41-52.
https://doi.org/10.1016/j.biotechadv.2014.12.006

Chen K., Wang Y., Zhang R., Zhang H., and Gao C., 2019, CRISPR/Cas genome editing and precision plant breeding in agriculture, Annual Review of Plant Biology, 70: 667-697.
https://doi.org/10.1146/annurev-arplant-050718-100049

Crossa J., Pérez-Rodríguez P., Cuevas J., Montesinos-López O., Jarquín D., Campos G., Burgueño J., González-Camacho J., Pérez-Elizalde S., Beyene Y., Dreisigacker S., Singh R., Zhang X., Gowda M., Roorkiwal M., Rutkoski J., and Varshney R., 2017, Genomic selection in plant breeding: methods, models, and perspectives, Trends in Plant Science, 22(11): 961-975.
https://doi.org/10.1016/j.tplants.2017.08.011

Dawei W., Yu L., Wei Z., and Zhouqi L., 2010, Establishment and optimization of AFLP reaction system in *Eucommia ulmoides* Oliv, Journal of Northwest AandF University, 38(6): 88-94.

Du Q., Wu Z., Liu P., Qing J., He F., Du L., Sun Z., Zhu L., Zheng H., Sun Z., Yang L., Wang L., and Du H., 2023, The chromosome-level genome of *Eucommia ulmoides* provides insights into sex differentiation and α-linolenic acid biosynthesis, Frontiers in Plant Science, 14: 1118363.
https://doi.org/10.3389/fpls.2023.1118363

Ge H., Walhout A., and Vidal M., 2003, Integrating 'omic' information: a bridge between genomics and systems biology, Trends in Genetics : TIG, 19(10): 551-560.
https://doi.org/10.1016/j.tig.2003.08.009

Goddard M., and Hayes B., 2007, Genomic selection, Journal of animal breeding and genetics = Zeitschrift fur Tierzuchtung und Zuchtungsbiologie, 124(6): 323-330.
https://doi.org/10.1111/j.1439-0388.2007.00702.x

Heslot N., Jannink J., and Sorrells M., 2015, Perspectives for genomic selection applications and research in plants, Crop Science, 55(1): 1-12.
https://doi.org/10.2135/cropsci2014.03.0249

Hu X., Li Y., Xia Y., and Ma Y., 2023, The MYB transcription factor family in *Eucommia ulmoides*: genome-wide identification, characterization, and network analysis in relation to the rubber biosynthetic genes, Forests. 14(10): 2064.
https://doi.org/10.3390/f14102064

Jin C., Li Z., Li Y., Wang S., Li L., and Liu M., 2020, Update of genetic linkage map and QTL analysis for growth traits in *Eucommia ulmoides* Oliver, Forests, 11(3): 311-311.
https://doi.org/10.3390/f11030311

Kumar R., Jorben J., Yadav R., and Senapati M., 2020, Genomics and its application in crop improvement, Journal of Pharmacognosy and Phytochemistry, 9(1): 547-552.
https://doi.org/10.22271/phyto.2020.v9.i1i.10488

Li Y., Liang J., Deng B., Jiang Y., Zhu J., Chen L., Li M., and Li J., 2023, Applications and prospects of CRISPR/Cas9-mediated base editing in plant breeding, Current Issues in Molecular Biology, 45(2): 918-935.
https://doi.org/10.3390/cimb45020059

Li Y., Wang D., Li Z., Wei J., Jin C., and Liu M., 2014, A molecular genetic linkage map of *Eucommia ulmoides* and quantitative trait loci (QTL) analysis for growth traits, International Journal of Molecular Sciences, 15(2): 2053-2074.
https://doi.org/10.3390/ijms15022053

Li Y., Wei H., Yang J., Du K., Li J., Zhang Y., Qiu T., Liu Z., Ren Y., Song L., and Kang X., 2020, High-quality de novo assembly of the *Eucommia ulmoides* haploid genome provides new insights into evolution and rubber biosynthesis, Horticulture Research, 7(1): 183.
https://doi.org/10.1038/s41438-020-00406-w

Liu C., Wang L., Lu W., Zhong J., Du H., Liu P., Du Q., Du L., and Qing J., 2022, Construction of SNP-Based high-density genetic map using genotyping by sequencing (GBS) and QTL analysis of growth traits in *Eucommia ulmoides* Oliv, Forests, 13(9): 1479-1479.
https://doi.org/10.3390/f13091479

Liu H., Fu J., Du H., Hu J., and Wuyun T., 2016, De novo sequencing of *Eucommia ulmoides* flower bud transcriptomes for identification of genes related to floral development, Genomics Data, 9: 105-110.
https://doi.org/10.1016/j.gdata.2016.07.001

Merrick L., Herr A., Sandhu K., Lozada D., and Carter A., 2022, Optimizing plant breeding programs for genomic selection, Agronomy. 12(3): 714-714.
https://doi.org/10.3390/agronomy12030714

Poland J., 2015, Breeding-assisted genomics, Current opinion in plant biology, 24: 119-124.
https://doi.org/10.1016/j.pbi.2015.02.009

Rao M., and Wang L., 2021, CRISPR/Cas9 technology for improving agronomic traits and future prospective in agriculture, Planta, 254(4): 68.
https://doi.org/10.1007/s00425-021-03716-y

Rodríguez-Leal D., Lemmon Z., Man J., Bartlett M., and Lippman Z., 2017, Engineering quantitative trait variation for crop improvement by genome editing, Cell, 171(2): 470-480.
https://doi.org/10.1016/j.cell.2017.08.030

Salgotra R., and Stewart C., 2020, Functional markers for precision plant breeding, International Journal of Molecular Sciences, 21(13): 4792.
https://doi.org/10.3390/ijms21134792

Tchounke B., Sanchez L., Bell J., and Cros D., 2022, Mate selection: A useful approach to maximize genetic gain and control inbreeding in genomic and conventional oil palm (Elaeis guineensis Jacq.) hybrid breeding, PLOS Computational Biology, 19(9): e1010290.
https://doi.org/10.1371/journal.pcbi.1010290

Varshney R., Graner A., and Sorrells M., 2005, Genomics-assisted breeding for crop improvement, Trends in Plant Science, 10(12): 621-630.
https://doi.org/10.1016/j.tplants.2005.10.004

Varshney R., Roorkiwal M., and Sorrells M., 2017, Genomic selection for crop improvement, Crop Science, 49(1): 1-12.
https://doi.org/10.2135/cropsci2008.08.0512

Wan L., Wang Z., Tang M., Hong D., Sun Y., Ren J., Zhang N., and Zeng H., 2021, CRISPR-Cas9 gene editing for fruit and vegetable crops: strategies and prospects, Horticulturae, 14(9): 1074.
https://doi.org/10.3390/horticulturae7070193

Wang B., Zhu L., Zhao B., Zhao Y., Xie Y., Zheng Z., Li Y., Sun J., and Wang H., 2019, Development of a haploid-inducer mediated genome editing system for accelerating maize breeding, Molecular Plant, 12(4): 597-602.
https://doi.org/10.1016/j.molp.2019.03.006

Wang D., Li Y., and Li Z., 2011, Identification of a male-specific amplified fragment length polymorphism (AFLP) and a sequence characterized amplified region (SCAR) marker in *Eucommia ulmoides* Oliv, International Journal of Molecular Sciences, 12(1): 857-864.
https://doi.org/10.3390/ijms12010857

Wang W., Chen S., and Zhang X., 2018, Whole-genome comparison reveals heterogeneous divergence and mutation hotspots in chloroplast genome of *Eucommia ulmoides* Oliver, International Journal of Molecular Sciences, 19(4): 1037.
https://doi.org/10.3390/ijms19041037

Wang W., Yang G., Deng X., Shao F., Li Y., Guo W., Liang H., and Zhang X., 2020, Molecular sex identification in the hardy rubber tree (*Eucommia ulmoides* Oliver) via ddRAD markers, International Journal of Genomics, pp.2420976.
https://doi.org/10.1155/2020/2420976

Weckwerth W., Ghatak A., Bellaire A., Chaturvedi P., and Varshney R., 2020, PANOMICS meets germplasm, Plant Biotechnology Journal, 18(7): 1507-1525.
https://doi.org/10.1111/pbi.13372

Zhang H., Zhang J., Lang Z., Botella J., and Zhu J., 2017, Genome editing-principles and applications for functional genomics research and crop improvement, Critical Reviews in Plant Sciences, 36(4): 291-309.
https://doi.org/10.1080/07352689.2017.1402989

Zhang S., Xu T., Ren Y., Song L., Liu Z., Kang X., and Li Y., 2023a, The NAC transcription factor family in *Eucommia ulmoides*: Genome-wide identification, characterization, and network analysis in relation to the rubber biosynthetic genes, Frontiers in Plant Science 14: 1030298. https://doi.org/10.3389/fpls.2023.1030298

Zhang X., Wang X., Pan L., Guo W., Li Y., and Wang W., 2023b, Genome-wide identification and expression analysis of MADS-box transcription factors reveal their involvement in sex determination of hardy rubber tree (*Eucommia ulmoides* oliv.), Frontiers in Genetics, 14: 1138703. https://doi.org/10.3389/fgene.2023.1138703