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Functional Study of Key Genes in *Eucalyptus* Asexual Reproduction

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Abstract Transcriptome analysis revealed significant differences in gene expression between differentiated and dedifferentiated tissues in *Eucalyptus* species with varying embryogenetic potentials. Specifically, 9 229 and 8 989 differentially expressed genes (DEGs) were identified in *E. camaldulensis* and *E. grandis x urophylla*, respectively. Key genes involved in somatic embryogenesis, such as somatic embryogenesis receptor kinase, ethylene, auxin, and transcription factors, were differentially regulated. Functional studies using *Eucalyptus* hairy roots demonstrated the utility of these genes in secondary cell wall biosynthesis and wood formation. Overexpression of FLOWERING LOCUS T (FT) induced early flowering, facilitating rapid breeding cycles. The identified genes and their functional roles offer valuable insights for improving vegetative propagation and breeding programs. The findings also highlight the potential of using genetic and transcriptomic tools to accelerate the development of *Eucalyptus* species with desirable traits. This study aimed to identify and functionally characterize key genes involved in the asexual reproduction of *Eucalyptus*, focusing on somatic embryogenesis and dedifferentiation processes and enhance the understanding of molecular mechanisms underlying these processes to improve vegetative propagation techniques for commercial and breeding purposes.

Keywords *Eucalyptus*; Asexual reproduction; Somatic embryogenesis; Gene expression; Vegetative propagation

1 Introduction

Eucalyptus, a genus within the Myrtaceae family, holds significant economic and ecological importance globally (Silva-Pando and Pino-Pérez, 2016). It is the most widely planted hardwood, valued for its rapid growth, adaptability, and utility in producing timber, pulp, and bioenergy (Xiao et al., 2020; Amanpreet and Rajesh, 2021). The genus is also notable for its high foliar concentrations of secondary metabolites, which play crucial roles in mediating interactions with herbivores and influencing ecosystem functions (Külheim et al., 2011; Brezáni and Karel, 2013; Nasr et al., 2019). The adaptability and economic value of *Eucalyptus* make it a pivotal genus in both forestry and agricultural sectors.

Asexual reproduction, particularly through somatic embryogenesis (SE), is a critical method for the vegetative propagation of *Eucalyptus*. SE allows for the large-scale production of genetically uniform plants, which is essential for commercial forestry operations (Xiao et al., 2020). The process involves the dedifferentiation of plant cells to a meristematic state, followed by their redifferentiation into somatic embryos. This method is advantageous for maintaining desirable traits and enhancing the efficiency of breeding programs. However, the molecular mechanisms underlying SE in *Eucalyptus* are not fully understood, necessitating further research to elucidate the key genes involved in this process (Pinto et al., 2013; Xiao et al., 2020). Besides, Previous studies have highlighted the importance of various transcription factors, protein domains, and secondary metabolites in the growth and reproductive diversification of *Eucalyptus* (Külheim et al., 2011; Soler et al., 2015; Kersting et al., 2015).

The aim of this study is to investigate key genes involved in eucalyptus asexual reproduction, with a focus on somatic embryogenesis, and contribute to improved breeding programs and sustainable management of eucalyptus plantations by providing a detailed understanding of the genetic and molecular basis of eucalyptus asexual reproduction. This study aims to identify differentially expressed genes (DEGs) that play a key role in dedifferentiation and redifferentiation. This study also provides a scientific basis for the systematic study of the genetic mechanism of eucalyptus asexual reproduction, and emphasizes its importance in forestry and agriculture.

2 Overview of Asexual Reproduction in Plants

2.1 Mechanisms of asexual reproduction in angiosperms

Asexual reproduction in angiosperms involves various mechanisms that allow plants to reproduce without the fusion of gametes. This process can occur through vegetative propagation, where new plants grow from parts of the parent plant such as stems, roots, or leaves (Hewitt, 2020). Key genes and protein domains play a significant role in these processes. For instance, the expansion of certain protein domains in *Eucalyptus* has been linked to tissue-specific expression and subfunctionalization, which are crucial for the specialization of reproduction and interactions with the environment (Kersting et al., 2015). Additionally, the overexpression of specific genes like FLOWERING LOCUS T (FT) can induce early flowering, which is a form of asexual reproduction that accelerates breeding and genetic studies (Klocko et al., 2016).

2.2 Advantages of asexual reproduction in forestry

Asexual reproduction offers several advantages in forestry, particularly for species like *Eucalyptus* that are vital for industrial purposes. One significant benefit is the ability to produce genetically identical offspring, ensuring uniformity in desirable traits such as growth rate, wood quality, and resistance to pests and diseases (Finkeldey and Hattemer, 2007). This uniformity is crucial for maintaining the consistency and quality of forestry products. Moreover, asexual reproduction can significantly reduce the time required for breeding programs. For example, the overexpression of *FT* genes in *Eucalyptus* can lead to precocious flowering, thereby speeding up the breeding cycle and enabling faster genetic improvements (Klocko et al., 2016). This method also allows for the rapid propagation of superior genotypes, ensuring that the best traits are perpetuated in subsequent generations.

2.3 Common methods of asexual reproduction in *Eucalyptus*

Eucalyptus species employ several common methods of asexual reproduction, including vegetative propagation techniques such as cuttings, grafting, and tissue culture. These methods are widely used to produce clones of elite trees with desirable traits (Assis, 2011). The genetic basis of these methods is often linked to the expansion and modulation of specific protein domains that facilitate tissue-specific expression and functional specialization (Kersting et al., 2015). Additionally, genetic engineering approaches, such as the overexpression of *FT* genes, have been developed to induce early flowering and enhance reproductive efficiency in *Eucalyptus* (Klocko et al., 2016). These methods not only improve the speed and efficiency of breeding programs but also ensure the propagation of high-quality, uniform plant material for forestry applications.

3 Key Genes Involved in Asexual Reproduction

3.1 Identification of key genes in *Eucalyptus*

The identification of key genes involved in asexual reproduction in *Eucalyptus* has been a focal point of recent research. One significant study utilized transcriptome analysis to identify differentially expressed genes (DEGs) during the somatic embryogenesis (SE) process in *Eucalyptus* species. The study by Xiao et al. (2020) identified several genes, including somatic embryogenesis receptor kinase, ethylene, auxin, ribosomal protein, zinc finger protein, heat shock protein, histone, cell wall-related, and transcription factor genes, which are crucial for the dedifferentiation process necessary for SE. Another study focused on the LEAFY (LFY) orthologue, *ELFY*, in *Eucalyptus*, demonstrating that its disruption via CRISPR-Cas9 led to significant alterations in floral development, indicating its critical role in reproductive processes (Elorriaga et al., 2021). Additionally, the *Eucalyptus* cinnamoyl-CoA reductase1 (*EgCCR1*) gene was identified as a key player in lignin biosynthesis, which is essential for secondary cell wall formation and overall plant structure, indirectly influencing asexual reproduction capabilities (Plasencia et al., 2016).

3.2 Functional roles of these genes in asexual reproductive processes

The functional roles of these identified genes are diverse and critical for the asexual reproductive processes in *Eucalyptus*. The somatic embryogenesis receptor kinase and other DEGs identified in the transcriptome analysis are involved in the dedifferentiation of plant cells, a crucial step for SE, which allows for the vegetative propagation of *Eucalyptus* (Xiao et al., 2020). The *ELFY* gene, when disrupted, leads to sterile indeterminate inflorescences, indicating its role in controlling floral organ identity and development, which is essential for managing gene flow and ensuring the stability of asexual reproduction (Elorriaga et al., 2021). The *EgCCR1* gene plays a pivotal role in lignin biosynthesis, which is vital for the structural integrity of the plant, thereby supporting the overall health and viability of asexually reproduced plants (Plasencia et al., 2016).

3.3 Techniques used to study gene function in *Eucalyptus*

Several advanced techniques have been employed to study the function of these key genes in *Eucalyptus*. Transcriptome sequencing was used to compare the gene expression profiles between differentiated and dedifferentiated tissues, allowing for the identification of DEGs involved in somatic embryogenesis (Xiao et al., 2020). CRISPR-Cas9 technology was utilized to create targeted mutations in the *ELFY* gene, providing insights into its role in floral development and reproductive processes (Elorriaga et al., 2021). Additionally, the development of *Eucalyptus* hairy roots as a model system has enabled medium-throughput functional studies, including gene expression analysis through RT-qPCR, promoter expression studies, and the modulation of endogenous gene expression (Plasencia et al., 2016). These techniques collectively contribute to a comprehensive understanding of the genetic mechanisms underlying asexual reproduction in *Eucalyptus*.

4 Case Studies: *Eucalyptus* Gene Function and Asexual Reproduction

4.1 Detailed analysis of specific genes and their impact on propagation

In the study of *Eucalyptus* asexual reproduction, specific genes play crucial roles in the process of somatic embryogenesis (SE). Transcriptome analysis of *Eucalyptus* species, such as *E. camaldulensis* and *E. grandis* × *urophylla*, has identified a significant number of differentially expressed genes (*DEGs*) during the dedifferentiation process (Figure 1), which is a key step for plant cells to become meristematic. For instance, in *E. camaldulensis*, 2 003 up-regulated and 1 958 down-regulated genes were identified, including genes related to somatic embryogenesis receptor kinase, ethylene, auxin, ribosomal proteins, zinc finger proteins, heat shock proteins, histones, cell wall-related proteins, and transcription factors (Xiao et al., 2020). These findings highlight the complex molecular changes that occur during SE and provide a valuable resource for understanding the genetic basis of asexual reproduction in *Eucalyptus*.

4.2 Success stories of genetic manipulation enhancing asexual reproduction

Genetic manipulation has shown promise in enhancing asexual reproduction in *Eucalyptus*. By targeting specific genes identified through transcriptome analysis, researchers have been able to improve the efficiency of somatic embryogenesis. For example, the up-regulation of genes such as somatic embryogenesis receptor kinase and ethylene-related genes has been associated with higher embryogenetic potential in *E. camaldulensis* compared to *E. grandis* × *urophylla* (Figure 2) (Xiao et al., 2020). These genetic modifications have led to more successful and efficient propagation methods, which are crucial for commercial applications in forestry and bioenergy production.

4.3 Comparative analysis with other species

Comparative analysis of gene function in *Eucalyptus* with other species provides insights into the unique aspects of its asexual reproduction. The expansion of protein domains, particularly those related to reproduction and biotic and abiotic interactions, has been a key feature in the adaptive radiation of *Eucalyptus*. Tandem duplication of genes has facilitated tissue-specific expression and subfunctionalization, which are critical for the specialization of reproductive functions in *Eucalyptus* (Kersting et al., 2015). This contrasts with other species where different mechanisms may be at play, highlighting the unique evolutionary pathways that *Eucalyptus* has taken to optimize its reproductive strategies.

In summary, the detailed analysis of specific genes, success stories of genetic manipulation, and comparative studies with other species underscore the complexity and uniqueness of asexual reproduction in *Eucalyptus*. These insights not only enhance our understanding of *Eucalyptus* biology but also pave the way for improved propagation techniques that can benefit commercial forestry and bioenergy sectors.

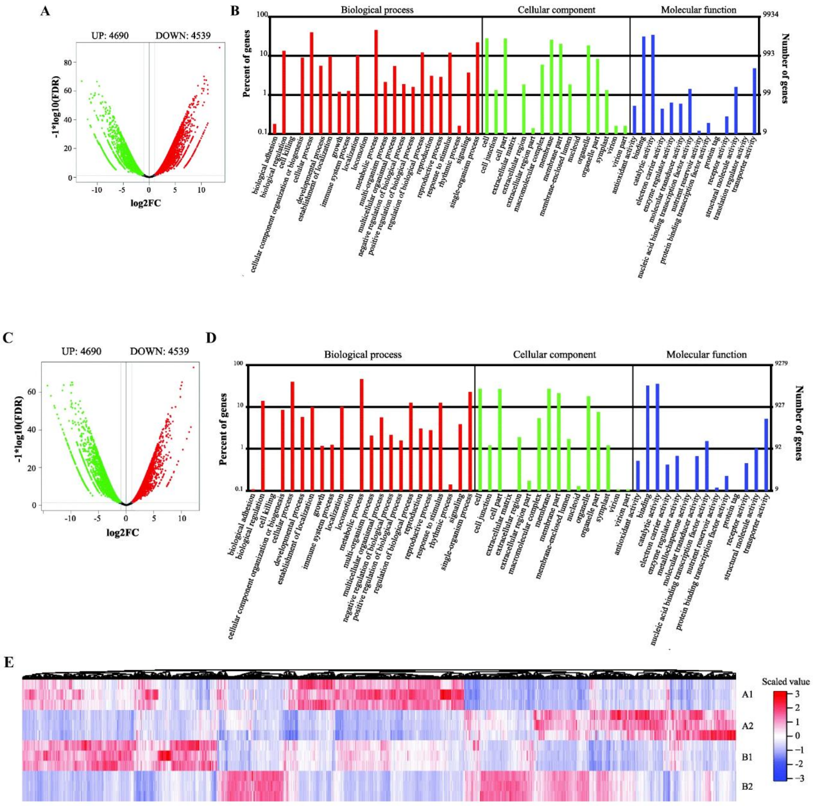

Figure 1 DEGs identified in the callus compared to the stem of *E. camaldulensis* and *E. grandis* × *urophylla* (Adopted from Xiao et al., 2020)

Image caption: A Volcano plot showing up- and down-regulated genes in the callus of *E. camaldulensis* compared to the stem. b Gene Ontology analysis for the DEGs identified in *E. camaldulensis*. c Volcano plot of the DEGs identified in the callus of *E. grandis* × *urophylla* compared to the stem. d Gene Ontology analysis of DEGs identified in *E. grandis* × *urophylla*. € Heat map of all DEGs identified in *E. camaldulensis* and *E. grandis* × *urophylla* showing the genes expression patterns in the dedifferentiation process (Adopted from Xiao et al., 2020)

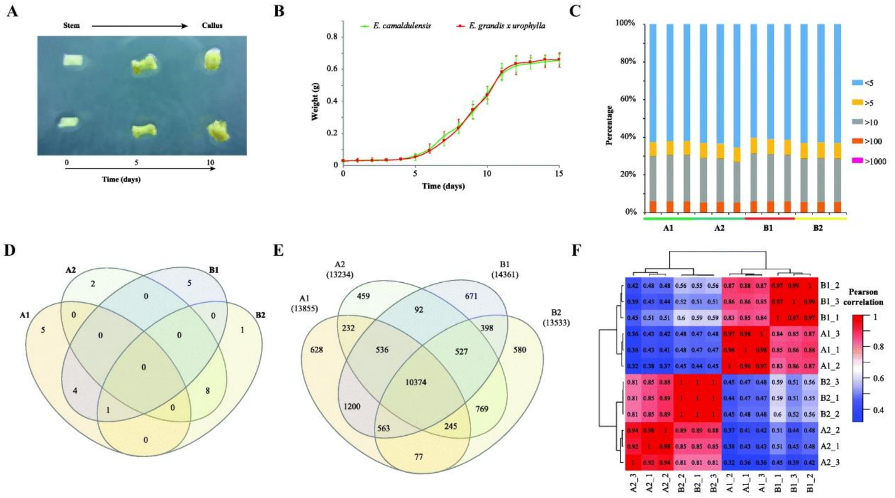

Figure 2 Morphological characterization of somatic callus and overview of the transcriptome sequencing (Adopted from Xiao et al., 2020)

Image caption: A Morphological characterization of the dedifferentiation from stem to callus. Upper panel: *E. camaldulensis*; lower panel: *E. grandis* × *urophylla*. b Growth curves of the callus tissue of *E. camaldulensis* and *E. grandis* × *urophylla*. c Number of genes identified in the stem and callus of *E. camaldulensis* (A1, A2) and *E. grandis* × *urophylla* (B1, B2). d Venn diagram of top 10 highly expressed genes in all samples. e Venn diagram of genes (TPM > 1) identified in all samples. f Heat map of sample correlation based on the gene expression profiles. Color bar represents the values of Pearson correlation (Adopted from Xiao et al., 2020)

5 Molecular Techniques in Genetic Studies

5.1 Advances in molecular biology relevant to *Eucalyptus* genetics

Recent advancements in molecular biology have significantly enhanced researchers' understanding of *Eucalyptus* genetics, particularly in the context of asexual reproduction. One notable study demonstrated that the overexpression of the FLOWERING LOCUS T (FT) gene in *Eucalyptus grandis* × *urophylla* hybrids can induce precocious flowering. This early flowering was achieved using various promoters, including the cauliflower mosaic virus 35S promoter and a heat-shock promoter, which facilitated the rapid onset of flowering within 1~5 months of transplanting. This technique not only accelerates the breeding process but also allows for the segregation of the transgene in progeny, thereby restoring normal growth and form in subsequent generations (Klocko et al., 2016).

5.2 Application of CRISPR/Cas9, RNAi, and other gene-editing tools

The application of gene-editing tools such as CRISPR/Cas9 and RNA interference (RNAi) has opened new avenues for functional studies of key genes in *Eucalyptus* asexual reproduction. These tools enable precise modifications of specific genes, allowing researchers to investigate their roles in various biological processes. For instance, CRISPR/Cas9 can be used to knock out or modify genes involved in flowering and growth, providing insights into their functions and potential applications in forestry (Nagle et al., 2023). RNAi, on the other hand, can be employed to silence genes temporarily, offering a reversible approach to study gene function without permanent genetic alterations.

5.3 Challenges and limitations in current research methodologies

Despite the promising advancements, several challenges and limitations persist in the current research methodologies. One major challenge is the complexity of the *Eucalyptus* genome, which can complicate gene-editing efforts and the interpretation of results (Myburg et al., 2014). Additionally, the long generation time of *Eucalyptus* trees poses a significant hurdle for breeding and genetic studies, although techniques like FT overexpression can mitigate this issue to some extent (Klocko et al., 2016).

Another limitation is the potential off-target effects of gene-editing tools such as CRISPR/Cas9, which can lead to unintended genetic modifications and phenotypic consequences. Furthermore, the efficiency of gene-editing techniques can vary, necessitating the development of optimized protocols for different *Eucalyptus* species and genetic backgrounds.

In conclusion, while molecular techniques have greatly advanced our understanding of *Eucalyptus* genetics, ongoing research is needed to address the challenges and limitations associated with these methodologies. By refining these techniques and developing new approaches, researchers can continue to uncover the genetic mechanisms underlying asexual reproduction in *Eucalyptus* and apply this knowledge to improve forestry practices.

6 Implications for *Eucalyptus* Breeding and Conservation

6.1 Enhancing propagation techniques through genetic insights

The functional study of key genes in *Eucalyptus* asexual reproduction has significant implications for enhancing propagation techniques. Overexpression of the *FLOWERING LOCUS T* (*FT*) gene has been shown to induce early flowering in *Eucalyptus*, which can accelerate breeding programs by reducing the time required for flowering and seed production (Jones et al., 2012; Klocko et al., 2016). This genetic modification allows for faster generation turnover, enabling more rapid selection and propagation of desirable traits. Additionally, transcriptome analysis has identified numerous genes involved in somatic embryogenesis, providing insights into the molecular mechanisms underlying vegetative propagation (Xiao et al., 2020). These findings can be leveraged to improve the efficiency and success rates of clonal propagation techniques, ensuring the consistent production of high-quality planting material.

6.2 Implications for biodiversity conservation and forestry practices

Understanding the genetic basis of key traits in *Eucalyptus* has profound implications for biodiversity conservation and sustainable forestry practices. The identification of genes associated with growth traits through genome-wide association studies (GWAS) enables the selection of genotypes that are better adapted to specific environmental conditions (Müller et al., 2018). This can enhance the resilience of *Eucalyptus* plantations to climate change and other environmental stresses. Furthermore, characterizing the adaptive genetic diversity in *Eucalyptus* populations helps in the conservation of genetic resources, ensuring the preservation of valuable alleles that contribute to the species' adaptability and survival (Dillon et al., 2014). Integrating genomic tools into breeding programs can also reduce the pressure on natural forests by increasing the productivity and sustainability of plantation forests (Grattapaglia, 2004; Grattapaglia and Kirst, 2008).

6.3 Future strategies for sustainable *Eucalyptus* cultivation

Future strategies for sustainable *Eucalyptus* cultivation will benefit from the integration of genomic information into breeding programs. The development of high-throughput genomic technologies allows for the detailed investigation of genes and their allelic variants that influence important traits such as wood properties, growth performance, and stress resistance (Grattapaglia, 2004; Gion et al., 2011). By linking phenotypes to specific genetic markers, breeders can make more informed decisions, leading to the development of superior *Eucalyptus* varieties with enhanced economic and ecological value. Additionally, the use of novel reference genes for gene expression normalization in *Eucalyptus* species ensures accurate and reliable expression profiling, which is crucial for functional genomics studies (Oliveira et al., 2011). These advancements will facilitate the implementation of precision breeding techniques, ultimately contributing to the sustainable management and conservation of *Eucalyptus* resources.

7 Integrating Genomic Data with Practical Forestry

7.1 From lab to field: applying genetic discoveries

The transition from laboratory genetic discoveries to practical forestry applications is a critical step in enhancing the productivity and sustainability of *Eucalyptus* plantations. Recent advancements in genomic technologies have provided unprecedented insights into the genetic basis of key traits such as growth, wood quality, and disease resistance. For instance, genome-wide association studies (GWAS) have identified significant genetic markers associated with growth traits in *Eucalyptus*, which can be utilized in breeding programs to select for superior genotypes (Müller et al., 2018). Additionally, the development of efficient transformation systems, such as the *Eucalyptus* hairy roots protocol, allows for the functional characterization of candidate genes involved in wood formation, thereby accelerating the application of genetic discoveries in the field (Plasencia et al., 2016).

7.2 Role of genomics in forestry management

Genomics plays a pivotal role in modern forestry management by enabling the precise selection and breeding of *Eucalyptus* trees with desirable traits. Genomic selection (GS) has emerged as a powerful tool to capture the 'missing heritability' of complex traits, thereby improving the accuracy and efficiency of breeding programs (Resende et al., 2012). The integration of genomic data into traditional breeding practices allows for the identification of quantitative trait loci (QTL) and the development of marker-assisted selection strategies, which can significantly enhance the genetic gains in *Eucalyptus* breeding (Grattapaglia and Kirst, 2008). Furthermore, the availability of comprehensive genomic resources, such as the EUCAWOOD dataset, provides valuable information on genes involved in wood formation, facilitating targeted breeding for improved wood properties (Rengel et al., 2009).

7.3 Case examples of genomic applications in forestry

Several case studies highlight the successful application of genomic data in *Eucalyptus* forestry. One notable example is the development of a high-density SNP chip for *Eucalyptus*, which enables genome-wide association studies and genomic selection across multiple species (Silva-Junior et al., 2015). This tool has been instrumental in identifying genetic variants associated with important traits, thereby informing breeding decisions and improving the genetic quality of *Eucalyptus* plantations. Another example is the use of the eCALIBRATOR tool to identify key genes and pathways involved in *Eucalyptus* defense against biotic stressors, providing insights into the genetic basis of disease resistance and informing the development of resistant genotypes (Figure 3) (Toit et al., 2020). These case studies demonstrate the practical benefits of integrating genomic data into forestry management, ultimately leading to more productive and resilient *Eucalyptus* plantations.

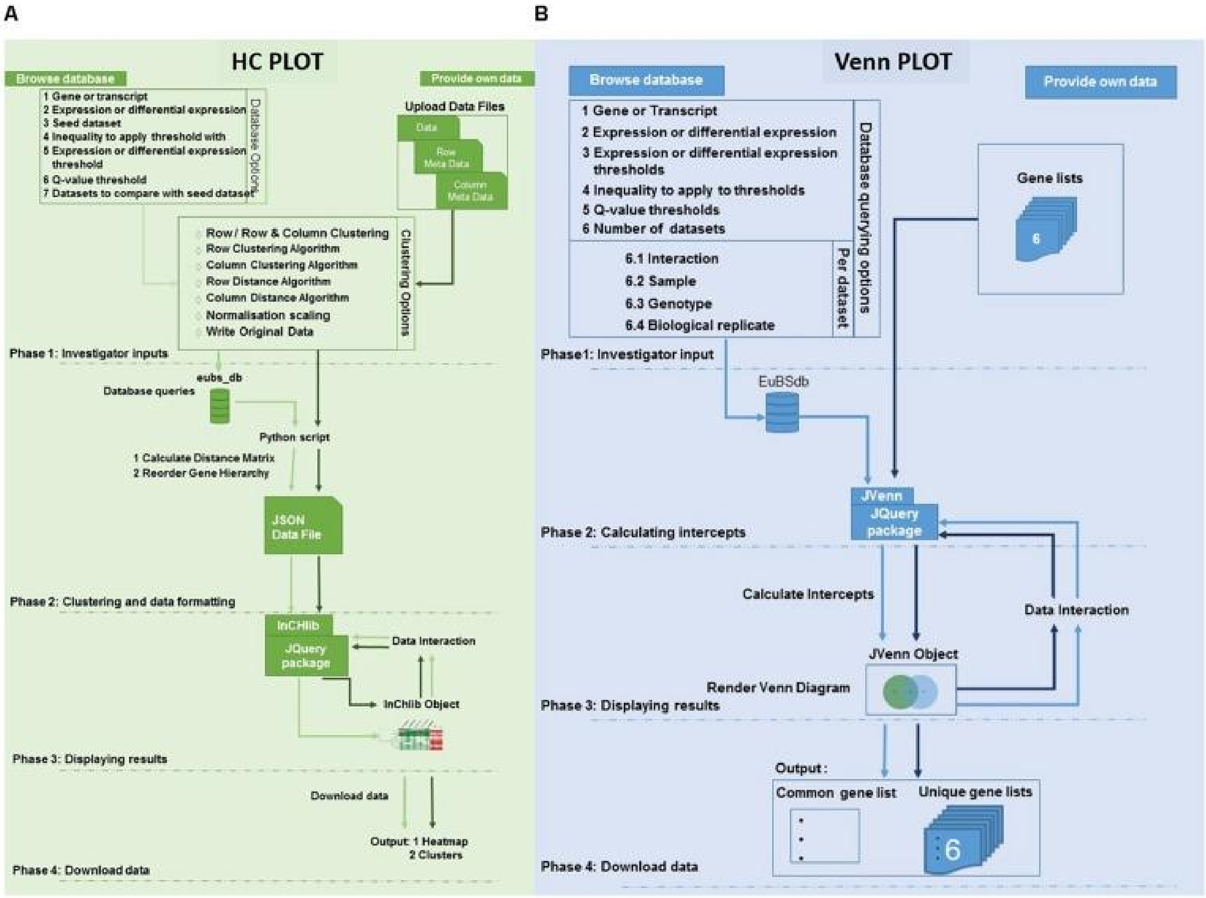

Figure 3 Architecture of the eCALIBRATOR tool (Adopted from Toit et al., 2020)

Image caption: (A) The flow of the HC Plot pipeline for the "PROVIDE OWN DATA" option is in dark green while the flow of the pipeline for the "BROWSE DATABASE" option is in light green. The pipeline is split into five different phases. PHASE 1: Investigator options specific to the data input method, PHASE 2: Clustering and data formatting, PHASE 3: Displaying results and PHASE 4: Download data. (B) The flow of the Venn Plot pipeline for the "PROVIDE OWN DATA" option is in dark blue while the flow of the pipeline for the "BROWSE DATABASE" option is in light blue. The pipeline is split into four different phases. PHASE 1: Investigator options specific to the data input method, PHASE 2: Calculating the intercepts required to generate the venn, PHASE 3: Displaying results and PHASE 4: Download data (Adopted from Toit et al., 2020)

8 Future Directions in *Eucalyptus* Genetic Research

8.1 Emerging trends and innovations in plant genetics

Recent advancements in plant genetics have significantly impacted the study of *Eucalyptus*, particularly in understanding the genetic mechanisms underlying asexual reproduction and adaptive traits (Xie et al., 2017). The comparative analysis of protein domain evolution in *Eucalyptus grandis* has revealed that tandem duplication of genes plays a crucial role in the expansion of protein domains, which are essential for reproductive specialization and interactions with biotic and abiotic factors (Kersting et al., 2015). Additionally, the overexpression of FLOWERING LOCUS T (FT) has been shown to induce early flowering in *Eucalyptus*, which can accelerate breeding programs and genetic studies (Klocko et al., 2016). These innovations highlight the potential for genetic modifications to enhance desirable traits in *Eucalyptus*.

8.2 Potential for new discoveries in *Eucalyptus* genome studies

The comprehensive genomic analysis of *Eucalyptus* species has opened new avenues for discovering genes involved in asexual reproduction and other critical traits. For instance, transcriptome analysis has identified numerous differentially expressed genes during the somatic embryogenesis of *Eucalyptus*, providing insights into the molecular mechanisms of dedifferentiation and embryogenesis (Xiao et al., 2020). Furthermore, studies on citrus have demonstrated the potential for identifying genetic loci responsible for asexual reproduction, which could be applied to *Eucalyptus* to improve vegetative propagation methods (Wang et al., 2017). These findings suggest that continued genomic studies will likely uncover new genes and pathways that can be targeted for genetic improvement.

8.3 Collaborative research initiatives and funding opportunities

To fully realize the potential of genetic research in *Eucalyptus*, collaborative research initiatives and funding opportunities are essential. International collaborations can facilitate the sharing of resources, expertise, and data, thereby accelerating the pace of discovery and application. Funding from governmental and private organizations can support large-scale genomic projects, such as the sequencing of diverse *Eucalyptus* species and the development of advanced breeding techniques. By fostering a collaborative research environment and securing adequate funding, the scientific community can drive significant advancements in *Eucalyptus* genetic research, ultimately benefiting forestry and agricultural industries worldwide.

9 Concluding Remarks

Transcriptome analysis revealed a substantial number of differentially expressed genes (DEGs) between differentiated and dedifferentiated tissues in *Eucalyptus* species with varying embryogenetic potentials. Specifically, 9 229 and 8 989 DEGs were identified in *E. camaldulensis* and *E. grandis* × *urophylla*, respectively, with a notable number of genes involved in key regulatory pathways such as somatic embryogenesis receptor kinase, ethylene, auxin, and various transcription factors. Additionally, the study highlighted the role of protein domain evolution and tandem duplication in the diversification and specialization of reproductive functions in *Eucalyptus*, further emphasizing the genetic complexity and adaptability of this genus. This study has advanced researchers' understanding of the genetic mechanisms underlying asexual reproduction in *Eucalyptus*, particularly through somatic embryogenesis (SE).

By elucidating the molecular mechanisms of somatic embryogenesis in *Eucalyptus*, this research provides a valuable resource for future genetic studies and breeding programs aimed at improving vegetative propagation techniques. The identification of stable reference genes for gene expression normalization across different *Eucalyptus* species and tissues enhances the accuracy and reliability of gene expression studies, facilitating more precise functional genomics research. Furthermore, understanding the evolutionary dynamics of protein domains in *Eucalyptus* offers insights into the adaptive strategies of this genus, which can inform conservation and management practices in forestry. The findings from this study contribute significantly to both plant genetic and forestry sciences.

Future research should focus on further characterizing the specific roles of the identified DEGs in the somatic embryogenesis process. Functional validation studies, such as gene knockouts or overexpression experiments, could provide deeper insights into the regulatory networks governing asexual reproduction in *Eucalyptus*. Additionally, expanding the transcriptome analysis to include more *Eucalyptus* species with varying degrees of embryogenetic potential could uncover broader genetic patterns and enhance the generalizability of the findings.

From a practical standpoint, the development of molecular markers based on the identified DEGs could improve the selection and breeding of *Eucalyptus* varieties with superior asexual reproduction capabilities. Moreover, the application of the stable reference genes identified in this study can enhance the precision of gene expression studies in *Eucalyptus*, leading to more effective genetic engineering and biotechnological interventions aimed at improving growth, stress resistance, and overall productivity in forestry.

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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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