Molecular Plant Breeding 2024, Vol.15, No.5, 317-327
http://genbreedpublisher.com/index.php/mpb

Review and Progress **Open Access**

Genome Editing Improvement Study of *Eucalyptus* Wood Quality Traits

Wenfang Wang ✉
Institute of Life Sciences, Jiyang College of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China
✉ Corresponding email: wenfang.wang@jicat.org

Molecular Plant Breeding, 2024, Vol.15, No.5 doi: 10.5376/mpb.2024.15.0030
Received: 22 Sep., 2024
Accepted: 23 Oct., 2024
Published: 31 Oct., 2024

Preferred citation for this article:
Wang W.F., 2024, Genome editing improvement study of *Eucalyptus* wood quality traits, Molecular Plant Breeding, 15(5): 317-327 (doi: 10.5376/mpb.2024.15.0030)

Abstract This study aims to evaluate the potential of genome editing technologies in improving wood quality traits of *Eucalyptus*, focusing on the current advancements of tools such as CRISPR/Cas9, base editing, and prime editing, and their application in *Eucalyptus* breeding programs. Significant progress has been identified in modifying lignin content and composition, enhancing cellulose content and fiber quality, and optimizing wood density and pulp yield using genome editing technologies. The results demonstrate the successful application of CRISPR/Cas9 targeting lignin biosynthesis genes, precise genetic modifications using base and prime editing, and the development of genomic selection models for predicting wood traits in *Eucalyptus*. Case studies highlight integrative approaches to simultaneously improve growth and wood quality traits, the use of regional heritability mapping to identify stable QTLs, and the implementation of genomic selection in breeding programs. The findings emphasize the transformative potential of genome editing in *Eucalyptus*, providing a pathway for efficient and sustainable improvement of wood quality traits. Integrating genome editing with traditional breeding methods and omics technologies can accelerate the development of superior *Eucalyptus* varieties. Future research should focus on advancing genome editing tools, conducting extensive field trials, and addressing ethical and regulatory issues to fully realize the potential benefits of these technologies.

Keywords *Eucalyptus*; Genome editing; CRISPR/Cas9; Wood quality traits; Genomic selection; QTL mapping

1 Introduction

Eucalyptus species are among the most widely planted hardwoods globally, renowned for their rapid growth, adaptability, and valuable wood properties. These trees are pivotal in the timber, pulp, and bioenergy industries, providing a renewable resource of fiber and energy. *Eucalyptus grandis*, for instance, has been extensively studied and sequenced, revealing a genome rich in genes for specialized metabolites and chemical defenses, which contribute to its economic value (Myburg et al., 2014). The adaptability and growth of *Eucalyptus* make it a crucial species for plantation forestry, reducing pressure on native forests and supporting biodiversity (Wang et al., 2021).

Traditional breeding methods for improving wood quality traits in *Eucalyptus* face significant limitations due to the species' genetic complexity and long breeding cycles (Thumma et al., 2010). Conventional breeding is slow and costly, often failing to capture the full heritability of complex traits such as wood density, lignin content, and pulp yield (Resende et al., 2012; Ballesta et al., 2019). The heterozygosity of *Eucalyptus* species further complicates germplasm improvement through crossbreeding (Wang et al., 2021). As a result, there is a pressing need for advanced genomic tools to enhance breeding efficiency and accuracy. Genomic selection (GS) and genome-wide association studies (GWAS) have shown promise in capturing the 'missing heritability' of these traits, providing a new perspective on quantitative trait variation and offering revolutionary tools for applied tree improvement (Resende et al., 2012; Ballesta et al., 2019).

Genome editing technologies, particularly CRISPR/Cas9, have emerged as powerful tools for precise genetic modifications in plants. CRISPR/Cas9 allows for targeted alterations in the genome, enabling the improvement of specific traits with high accuracy. This technology has been successfully applied in *Eucalyptus* to enhance wood quality and increase plantation productivity. For instance, a fluorescence labeling method using CRISPR/Cas9 has been developed to obtain positive transformed progenies, facilitat*ing the efficient genetic transformation of Eucalyptus* (Wang et al., 2021). The application of genome editing in *Eucalyptus* breeding programs holds significant potential for accelerating the development of superior germplasm with desirable wood quality traits (Plasencia et al., 2016; Ballesta et al., 2018).

This study is to explore the potential of gene editing technology in improving the quality characteristics of *Eucalyptus* wood. It involves a comprehensive understanding of genomic strategies employed to enhance *Eucalyptus* wood quality, identifying potential genetic targets for future research, and gaining deep insights into the practical applications of gene editing in forestry. By thoroughly analyzing how modern genomic tools can revolutionize *Eucalyptus* breeding programs, the study aims to ultimately promote more efficient and sustainable forestry practices, thereby contributing to the field.

2 Genome Editing Technologies for Wood Quality Improvement

2.1 CRISPR/Cas9 system

The CRISPR/Cas9 system has emerged as a groundbreaking technology in genetic engineering, enabling precise modifications of the genome. The CRISPR/Cas9 system has revolutionized genome editing due to its simplicity, efficiency, and versatility (Liu et al., 2021; Min et al., 2022). This technology relies on the complementarity of a guide RNA (gRNA) to a specific DNA sequence and the endonuclease activity of the Cas9 protein, which introduces double-stranded breaks at the target site. These breaks can be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR), leading to targeted mutations or precise gene modifications (Bortesi and Fischer, 2015; Arora and Narula, 2017; Chen et al., 2019).

In *Eucalyptus*, the CRISPR/Cas9 system has been successfully implemented to study wood-related genes. For instance, Dai et al. (2020) demonstrated the use of CRISPR/Cas9 in *Eucalyptus* hairy roots to target genes involved in lignin biosynthesis and auxin signaling. The study showed that editing the *Cinnamoyl-CoA Reductase1* (*CCR1*) gene resulted in decreased lignification and altered wood properties, highlighting the potential of CRISPR/Cas9 for functional genomics and wood quality improvement in *Eucalyptus*.

2.2 Genome editing techniques: base editing and prime editing

Recent advancements in genome editing have led to the development of base editing and prime editing technologies, which allow for precise single-nucleotide modifications without introducing double-stranded breaks. Base editors use a modified Cas9 nickase fused to a deaminase enzyme to convert specific DNA bases, while prime editors use a reverse transcriptase to directly write new genetic information into the DNA (Li et al., 2021; Molla et al., 2021; Wang et al., 2021b).

These precision editing tools have been applied to various plant species, including woody plants, to achieve targeted nucleotide substitutions with high efficiency and minimal off-target effects. Molla et al. (2021) analyzed the current status of base and prime editors in plants, emphasizing their potential for precise genome modifications and their applications in improving traits such as wood quality in *Eucalyptus*.

2.3 Delivery methods for genome editing

Efficient delivery of genome editing tools into plant cells is crucial for the success of genetic modifications. Several methods have been developed to introduce CRISPR/Cas9 components, base editors, and prime editors into *Eucalyptus* cells, including Agrobacterium-mediated transformation, biolistic particle delivery, and protoplast transfection (Arora and Narula, 2017; Chen et al., 2019; Wang et al., 2021a).

Wang et al. (2021a) described an efficient genetic transformation method for *Eucalyptus* using CRISPR/Cas9 technology. The study established a fluorescence labeling system to identify successfully transformed progenies, facilitating the selection of edited plants. This method can be used to improve *Eucalyptus* germplasm and enhance wood quality traits through precise genome editing.

3 Genetic Basis of Wood Quality Traits

3.1 Quantitative trait loci (QTL) mapping

Quantitative trait loci (QTL) mapping is a powerful tool for identifying the genetic regions associated with complex traits. QTL mapping has been instrumental in identifying the genetic basis of wood quality traits in *Eucalyptus*. For instance, a study on *E.urophylla* × *tereticornis* hybrids identified 108 QTLs related to growth and wood property traits, with only a few showing stability across different environments. Notably, the QTL qSG10Stable, associated with the lignin syringyl-to-guaiacyl ratio, was stable across all tested environments, explaining a significant portion of phenotypic variation (Figure 1) (Zhu et al., 2023). These findings provide an important basis for the genetic improvement and molecular breeding of economic traits in *Eucalyptus*.

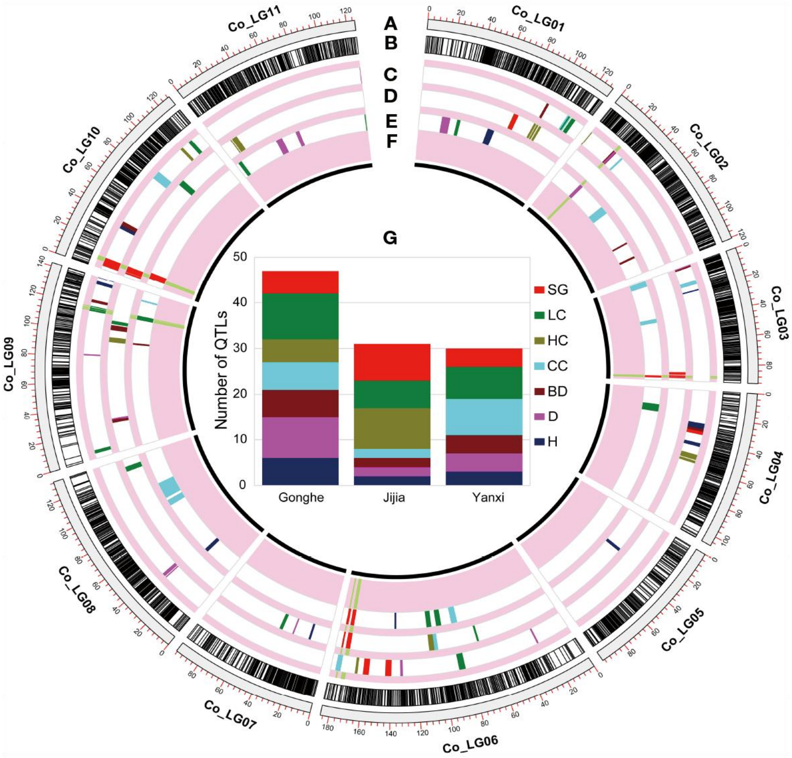

Figure 1 QTL mapping in multiple environments (Adopted from Zhu et al., 2023)

Image caption: (A) are the genetic distance in cM. (B) are the distribution of all loci in the consensus map. And the distribution of QTLs of growth and wood properties detected in Gonghe (C), Jijia (D) and Yanxi (E) environments. (F) are stable QTLs. (G) are QTL statistics for each trait at each environment (Adopted from Zhu et al., 2023)

Another study on *Eucalyptus globulus* identified five QTLs associated with intumescence severity, demonstrating the genetic basis for this trait and providing a framework for further investigation (Ammitzboll et al., 2018). Additionally, Bayesian mapping in *Eucalyptus cladocalyx* revealed large-effect pleiotropic QTLs for wood density and slenderness index, highlighting the potential for marker-assisted breeding in low-rainfall environments (Valenzuela et al., 2021).

3.2 Genomic selection and prediction

Genomic selection (GS) is an advanced breeding approach that leverages genome-wide marker information to predict the genetic potential of individuals for complex traits (Ballesta et al., 2018). Unlike traditional selection methods, which rely on phenotypic data alone, GS uses dense genetic markers distributed across the genome to build predictive models. These models can accurately estimate the breeding values of individuals, thereby accelerating the selection process and improving the efficiency of breeding programs.

Genomic selection (GS) models have shown promise in predicting wood quality traits in *Eucalyptus*. In *Eucalyptus globulus*, various GS methods, including Ridge Regression-BLUP (RRBLUP) and supervised principal component regression (PCR), demonstrated high predictive ability for traits such as branching quality and stem straightness (Ballesta et al., 2018). Similarly, in *Eucalyptus benthamii*, genomic relationship-based models like GBLUP and HBLUP provided more accurate predictions of wood density and tree volume by capturing hidden relatedness and correcting pedigree errors (Paludeto et al., 2021). The inclusion of haplotype effects in Bayesian genomic models further improved the predictive ability for low-heritability traits, suggesting a significant advantage for implementing GS in *Eucalyptus* breeding programs (Ballesta et al., 2019)

3.3 Transcriptome analysis

Transcriptome analysis involves the comprehensive study of RNA transcripts produced by the genome under specific conditions or developmental stages. This approach provides valuable insights into gene expression patterns and regulatory networks influencing various traits, including wood quality. In *Eucalyptus*, transcriptome studies have been instrumental in uncovering the molecular mechanisms underlying wood formation and quality.

Transcriptome analysis has provided valuable insights into the genes influencing wood quality in *Eucalyptus*. In a study on *Eucalyptus urophylla* × *E. tereticornis* hybrids, transcriptome analysis combined with QTL mapping identified 1 052 candidate genes associated with wood formation, including transcription factor families like TALE, which play a crucial role in secondary growth and wood formation (Zhu et al., 2023). These findings underscore the importance of integrating transcriptome data with QTL mapping to elucidate the regulatory mechanisms underlying wood quality traits. This approach can significantly enhance the understanding of genetic control over wood properties and inform targeted genome editing strategies for wood quality improvement.

4 Applications of Genome Editing in *Eucalyptus*

4.1 Improving lignin content and composition

Lignin is a complex polymer that provides structural integrity to plant cell walls but can pose challenges in industrial processing, particularly in pulp and paper production (Chanoca et al., 2019). High lignin content and certain lignin compositions make wood harder to process and less desirable for these applications. Genome editing technologies, particularly CRISPR/Cas9, have been utilized to target and modify genes involved in lignin biosynthesis to improve wood quality.

Genome editing has shown significant potential in modifying lignin content and composition in *Eucalyptus*, which is crucial for improving wood quality. The CRISPR/Cas9 technology has been effectively used to target key lignin biosynthetic genes such as *Cinnamoyl-CoA Reductase1* (*CCR1*). Editing these genes has resulted in decreased lignification and altered lignin composition, which are beneficial for reducing biomass recalcitrance and enhancing wood processing efficiency (Chanoca et al., 2019; Dai et al., 2020). Additionally, the overexpression of specific transcription factors like EgNAC141 has been found to positively regulate lignin biosynthesis, leading to increased lignin deposition and improved wood properties (Sun et al., 2021).

4.2 Enhancing cellulose content and fiber quality

Cellulose is a primary component of plant cell walls and a critical determinant of wood quality, particularly for its applications in paper, textiles, and biofuels. Enhancing cellulose content and improving fiber quality can significantly boost the economic value of *Eucalyptus* wood. Genome editing tools offer precise methods to modify genes involved in cellulose biosynthesis and regulation.

Enhancing cellulose content and fiber quality in *Eucalyptus* through genome editing involves targeting genes that regulate cellulose biosynthesis. Studies have shown that perturbing the expression of genes involved in the lignin biosynthetic pathway can also impact cellulose content and fiber quality. For instance, a multi-omics integrative analysis has demonstrated that modifying the expression of specific monolignol genes can lead to improvements in cellulose content and other wood traits (Wang et al., 2018). Furthermore, the use of advanced genetic transformation methods, such as the fluorescence labelling method with CRISPR/Cas9, has facilitated the efficient selection of genetically modified progenies with enhanced cellulose content and fiber quality (Wang et al., 2021).

4.3 Optimizing wood density and pulp yield

Optimizing wood density and pulp yield in *Eucalyptus* is another critical application of genome editing. Genetic parameters for wood density and other wood properties have been extensively studied, revealing that these traits have strong genetic control and can be effectively targeted through genome editing (Lima et al., 2019). The study found that the EgNAC141 transcription factor from *Eucalyptus* can significantly increase lignin deposition and wood density. Overexpression of EgNAC141 in Arabidopsis resulted in stronger lignification, larger xylem, and higher lignin content (Figure 2). Dual-luciferase reporter gene analysis showed that *EgNAC141* could activate the expression of lignin biosynthesis genes in Arabidopsis. These results indicate that *EgNAC141* is a positive regulator of lignin biosynthesis (Sun et al., 2021). Dai et al. (2020) targeted genes related to wood formation, such as *Cinnamoyl-CoA Reductase1* (*CCR1*), in *Eucalyptus* using CRISPR/Cas9 technology. They found that the edited *Eucalyptus* exhibited changes in lignin content and wood density, further validating the potential of gene editing in regulating wood properties.

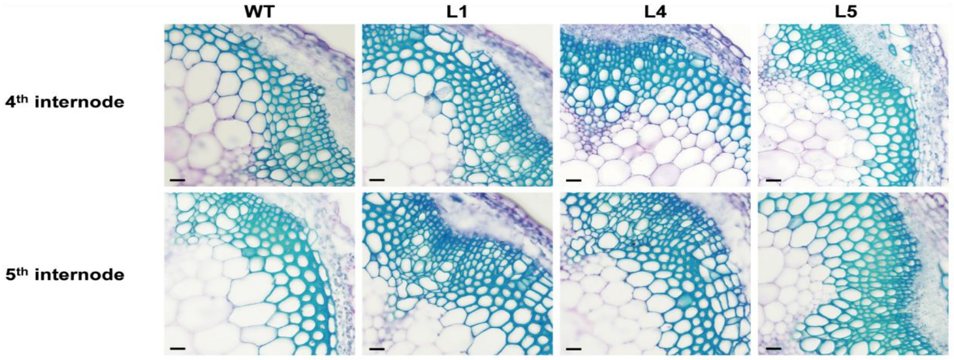

Figure 2 Microscopic analyses of stems from the control and *EgNAC141*-overexpressing Arabidopsis plants (Adopted from Sun et al., 2021)

Image caption: General view of the stem vascular tissues stained by TBO in the 4th and 5th internode of 2-month-old inflorescence stem transverse sections: WT and *EgNAC141*-overexpressing plants (L1, L4, and L5). Each independent line has four replicates, and the micrographs are from biological replicate 1 (R1). Bars: 500 µm (Adopted from Sun et al., 2021)

Furthermore, the study conducted a genome-wide marker relationship matrix analysis on *Eucalyptus* hybrids (*E. grandis* × *E. urophylla*), revealing that the chemical properties and density of wood are strongly genetically controlled and can be effectively improved through gene editing technologies (Lima et al., 2019). The integration of transcriptome and proteome data enabled the identification of key genes and regulatory mechanisms affecting wood density and pulp yield, further supporting the use of gene editing in these applications (Paludeto et al., 2021).

Genome editing offers promising avenues for improving lignin content and composition, enhancing cellulose content and fiber quality, and optimizing wood density and pulp yield in *Eucalyptus*. These advancements not only contribute to better wood quality but also support the sustainable and efficient use of *Eucalyptus* in various industrial applications.

5 Case Studies

5.1 Improving growth traits and wood quality simultaneously

Integrative approaches to enhance both growth and wood quality traits in *Eucalyptus* have shown promising results. For instance, a study on *Eucalyptus urograndis* hybrid demonstrated that genetic parameters for growth and wood properties can be effectively estimated using genome-wide SNP-based relationships. This approach allows for the simultaneous selection of growth and wood traits with minimal negative impact on genetic gain for growth, thanks to low correlations between these traits (Lima et al., 2019). Additionally, the use of genomic prediction models, such as single-step GBLUP, has been shown to improve the accuracy and bias of breeding value predictions by incorporating phenotypic data from non-genotyped trees, further enhancing the selection process for both growth and wood quality traits (Cappa et al., 2019).

5.2 Regional heritability mapping in *Eucalyptus*

Regional Heritability Mapping (RHM) has been applied to identify stable quantitative trait loci (QTL) across different environments. A study involving 3 373 individuals from four breeding populations used RHM in combination with single-marker GWAS and joint GWAS to detect loci significantly associated with growth traits. The study found that single SNP GWAS analyses detected only a few significant associations, but using gene-based and regional joint GWAS models, researchers identified multiple significantly associated genes and regions (Figure 3) (Müller et al., 2018). For instance, the gene-based joint GWAS revealed nine genes significantly associated with tree height across the four *Eucalyptus* breeding populations. These genes are primarily involved in cell wall biosynthesis and lignification processes, which are crucial for tree growth and wood quality. Furthermore, the joint GWAS method demonstrated repeated independent detection of the same SNP associations across different populations, providing unprecedented validation results for GWAS in forest trees. This approach has provided unprecedented validation results in forest trees, identifying genes involved in cell wall biosynthesis and lignification, which are critical for wood quality.

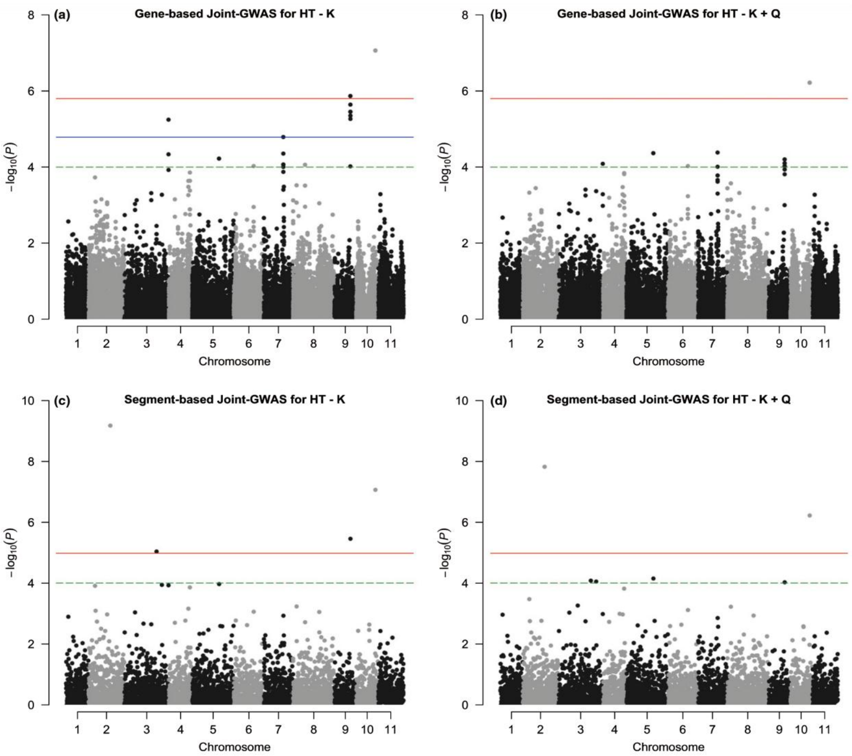

Figure 3 Manhattan plots of the associations for total height (HT) using gene-based (31 770 genes) and segment-based (4 766 windows) joint genome-wide association study (Joint-GWAS) for the combined dataset using four unrelated *Eucalyptus grandis* × *E. urophylla* hybrid breeding populations (Adopted from Müller et al., 2018)

Image caption: (a) Gene-based Joint-GWAS adjusted for kinship matrix, age of measurements and population of origin. (b) Gene-based Joint-GWAS adjusted for all covariates mentioned before with the inclusion of population structure. (c) Segment-based Joint-GWAS adjusted for kinship matrix, age of measurements and population of origin. (d) Segment-based Joint-GWAS adjusted for all other covariates with the inclusion of population structure. Red line indicates Bonferroni-corrected threshold with an experimental type I error rate at $\alpha = 0.05$, blue line indicates a false discovery rate (FDR) at 5% and green dashed line represents an *ad hoc* threshold of $-\log_{10}(P) = 4.0$ (Adopted from Müller et al., 2018)

5.3 Genomic selection models for wood traits

Implementing genomic selection models for predicting wood traits in *Eucalyptus* breeding programs has shown significant potential. For example, a study on *Eucalyptus benthamii* used various genomic models, including GBLUP and HBLUP, to estimate genetic parameters for lignin, extractives, carbohydrates, and wood density (Paludeto et al., 2021). The study found that genomic models provided more accurate predictions of trait values compared to pedigree-based models, with considerable dominance variance observed for all traits. This highlights the importance of considering non-additive genetic effects in genomic selection to improve overall selection efficiency.

Another study on *Eucalyptus globulus* indicates that genomic prediction models such as Ridge Regression-BLUP (RRBLUP) and supervised PCR have high predictive ability for growth and wood quality traits, further supporting the use of genomic selection in breeding programs (Ballesta et al., 2018). The study assessed various GS methods, including RRBLUP, Bayes-A, Bayes-B, BLASSO, and PCR, using a chip containing 60 000 single nucleotide polymorphism (SNP) markers. The research found that RRBLUP-B and supervised PCR models exhibited the highest predictive ability for most traits studied, particularly for branching quality (PA approximately 0.7) and growth traits (PA values ranging from 0.43 to 0.54). The results demonstrate the potential application of genomic prediction methods in early tree growth, offering new breeding strategies for forest planting in southern Chile, which could benefit the local economy.

6 Future Perspectives and Challenges

6.1 Ethical and regulatory considerations

The application of genome editing in forestry, particularly in *Eucalyptus* species, raises significant ethical and regulatory questions. The potential for transgene dispersal in natural forests is a major concern, as it could lead to unintended ecological consequences and affect biodiversity (Thapliyal et al., 2022). Regulatory frameworks need to be established to ensure that genome-edited trees are safe for the environment and do not pose risks to native species.

Regulatory frameworks governing the use of genome editing in plants vary widely across countries. In some regions, such as the European Union, strict regulations classify genome-edited organisms similarly to traditional genetically modified organisms (GMOs), necessitating rigorous risk assessments and approval processes (Eckerstorfer et al., 2023). In contrast, other regions, such as the United States, may have more lenient regulatory requirements for genome-edited crops that do not involve transgenic modifications.

As genome editing technologies continue to evolve, it is crucial to engage in transparent, science-based discussions with stakeholders, including policymakers, scientists, environmental groups, and the public. Establishing clear guidelines and robust regulatory frameworks will be essential to ensure the responsible use of genome editing in forestry. Additionally, public perception and acceptance of genetically modified trees must be considered, as societal resistance could hinder the adoption of these technologies (Thapliyal et al., 2022). Ethical considerations also include the potential socioeconomic impacts on communities dependent on traditional forestry practices, necessitating a balanced approach that weighs the benefits of improved wood quality and climate resilience against possible risks and public concerns.

6.2 Technological advances and integration

The future of genome editing in *Eucalyptus* improvement is closely tied to ongoing technological advancements and their integration with traditional breeding methods. Recent innovations, such as CRISPR/Cas systems with enhanced specificity, base editing, and prime editing, offer unprecedented precision and efficiency in genetic modifications (Dai et al., 2020; Cao et al., 2022). These technologies can target multiple genes simultaneously, enabling more comprehensive trait improvements.

For instance, the use of CRISPR/Cas9 has already shown success in targeting specific wood-related genes in *Eucalyptus*, leading to significant changes in lignin content and wood density (Dai et al., 2020). The integration of genome editing with genomic selection (GS) can further accelerate breeding cycles and increase genetic gains, as demonstrated in studies on *Eucalyptus grandis* and *Eucalyptus benthamii* (Mphahlele et al., 2020; Paludeto et al., 2021). By combining these advanced techniques, it is possible to achieve rapid and targeted improvements in wood quality traits, thereby enhancing the overall productivity and sustainability of *Eucalyptus* plantations.

6.3 Potential for climate resilience

Climate change poses significant challenges to forestry, including increased susceptibility to pests and diseases, extreme weather events, and shifting growing conditions. Genome editing holds substantial potential for developing climate-resilient *Eucalyptus* species that can withstand these challenges. Genome editing offers a powerful tool for developing climate-resilient *Eucalyptus* species. The ability to precisely modify genes associated with drought and pest resistance can significantly enhance the adaptability of *Eucalyptus* to changing climatic conditions.

For example, CRISPR-based technologies have been used to identify and edit genes related to drought tolerance and pest resistance, facilitating the selection of suitable gene editing targets for improving forest sustainability (Cao et al., 2022). Additionally, the genetic improvement of *Eucalyptus* for climate resilience can help mitigate the adverse effects of climate change on forest ecosystems, ensuring the continued availability of high-quality wood resources (Thapliyal et al., 2022). Paludeto et al. (2021) discussed the role of genome editing in enhancing the resilience of *Eucalyptus* to climate stressors. By targeting genes associated with drought tolerance, heat resistance, and pest and disease resistance, researchers can create *Eucalyptus* varieties better adapted to changing environmental conditions. For example, modifying genes involved in stress-responsive pathways, such as those regulating abscisic acid (ABA) signaling and heat shock proteins, can enhance the ability of *Eucalyptus* to cope with water scarcity and high temperatures. By harnessing the potential of genome editing, it is possible to create *Eucalyptus* species that are better equipped to withstand environmental stresses, thereby contributing to the long-term sustainability and productivity of forest plantations.

While genome editing offers transformative potential for improving *Eucalyptus* wood quality traits and developing climate-resilient varieties, it is essential to navigate the ethical and regulatory landscape carefully. Continued technological advancements and the integration of genome editing with traditional breeding methods will drive future innovations in forestry. By addressing these challenges and harnessing the full potential of genome editing, we can achieve significant progress in sustainable forestry and climate resilience.

7 Concluding Remarks

The advancements in genome editing, particularly through CRISPR/Cas9 technology, have shown significant potential in improving the wood quality traits of *Eucalyptus* species. The establishment of efficient genetic transformation methods has enabled the precise editing of specific genes, leading to enhanced wood properties. For instance, the use of CRISPR/Cas9 in *Eucalyptus* hairy roots demonstrated the ability to target and edit wood-related genes, resulting in decreased lignification and improved wood quality traits. Additionally, the implementation of fluorescence labelling methods has facilitated the identification of positive transformed progenies, further streamlining the genetic improvement process.

Genomic selection (GS) has also emerged as a powerful tool in *Eucalyptus* breeding programs. Studies have shown that GS can significantly reduce breeding cycle times and increase the accuracy of predicting wood quality traits. For example, the use of SNP and haplotype-based genomic models has improved the predictive ability for traits such as wood density and stem straightness. Moreover, integrating genome-wide association studies (GWAS) with GS has enhanced the identification of genetic markers associated with desirable wood properties, thereby improving the overall efficiency of breeding programs.

Future research should focus on optimizing genome editing techniques to achieve higher editing efficiencies and broader applicability across different *Eucalyptus* species. Enhancing the precision and efficiency of CRISPR/Cas9 technology will be crucial for the functional characterization of additional wood-related genes and the development of superior *Eucalyptus* varieties with improved wood quality traits.

Moreover, expanding the use of genomic selection in *Eucalyptus* breeding programs will be essential. This includes the integration of more comprehensive genomic data, such as transcriptomic and epigenomic information, to better understand the genetic architecture of wood quality traits. Additionally, the development of cost-effective and high-throughput genotyping methods will facilitate the implementation of GS on a larger scale, thereby accelerating the breeding process. Addressing the challenges posed by climate change, such as increased drought and temperature fluctuations, will be critical as well. Research should aim to identify and incorporate genetic markers associated with stress tolerance into breeding programs. This will ensure the development of resilient *Eucalyptus* varieties capable of maintaining high wood quality under adverse environmental conditions.

In conclusion, the future of genome editing in *Eucalyptus* holds immense potential for advancing wood quality traits and contributing to sustainable forestry practices. Continued research and innovation, coupled with responsible ethical and regulatory considerations, will drive the field forward, enabling the development of superior *Eucalyptus* varieties that meet the demands of industry and environmental sustainability.

Acknowledgments

GenBreed Publisher appreciates the timely feedback provided by the two anonymous peer reviewers on the manuscript of this study.

Conflict of Interest Disclosure

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

References

Ammitzboll H., Vaillancourt R., Potts B., Singarasa S., Mani R., and Freeman J., 2018, Quantitative trait loci (QTLs) for intumescence severity in *Eucalyptus globulus* and validation of QTL detection based on phenotyping using open-pollinated families of a mapping population, Plant Disease, 102(8): 1566-1573.
https://doi.org/10.1094/PDIS-01-18-0003-RE
PMid:30673414

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
PMid:29167680 PMCid:PMC5682324

Ballesta P., Maldonado C., Pérez-Rodríguez P., and Mora F., 2019, SNP and haplotype-based genomic selection of quantitative traits in *Eucalyptus globulus*, Plants, 8(9): 331.
https://doi.org/10.3390/plants8090331
PMid:31492041 PMCid:PMC6783840

Ballesta P., Serra N., Guerra F., Hasbún R., and Mora F., 2018, Genomic prediction of growth and stem quality traits in *Eucalyptus globulus* Labill. at its southernmost distribution limit in Chile, Forests, 9(12): 779.
https://doi.org/10.3390/f9120779

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
PMid:25536441

Cao H., Vu G., and Gailing O., 2022, From genome sequencing to CRISPR-based genome editing for climate-resilient forest trees, International Journal of Molecular Sciences, 23(2): 966.
https://doi.org/10.3390/ijms23020966
PMid:35055150 PMCid:PMC8780650

Cappa E., Lima B., Silva-Junior O., Garcia C., Mansfield S., and Grattapaglia D., 2019, Improving genomic prediction of growth and wood traits in *Eucalyptus* using phenotypes from non-genotyped trees by single-step GBLUP, Plant Science, 284: 9-15.
https://doi.org/10.1016/j.plantsci.2019.03.017
PMid:31084883

Chanoca A., Vries L., and Boerjan W., 2019, Lignin engineering in forest trees, Frontiers in Plant Science, 10: 912.
https://doi.org/10.3389/fpls.2019.00912
PMid:31404271 PMCid:PMC6671871

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
PMid:30835493

Dai Y., Hu G., Dupas A., Medina L., Blandels N., Clemente H., Ladouce N., Badawi M., Hernandez-Raquet G., Mounet F., Grima-Pettenati J., and Cassan-Wang H., 2020, Implementing the CRISPR/Cas9 technology in *Eucalyptus* hairy roots using wood-related genes, International Journal of Molecular Sciences, 21(10): 3408.
https://doi.org/10.3390/ijms21103408
PMid:32408486 PMCid:PMC7279396

Eckerstorfer M., Dolezel M., Engelhard M., Giovannelli V., Grabowski M., Heissenberger A., Lener M., Reichenbecher W., Simon S., Staiano G., Saucy A., Zünd J., and Lüthi C., 2023, Recommendations for the assessment of potential environmental effects of genome-editing applications in plants in the EU, Plants, 12(9): 1764.
https://doi.org/10.3390/plants12091764
PMid:37176822 PMCid:PMC10180588

Li C., Brant E., Budak H., and Zhang B., 2021, CRISPR/Cas: a Nobel Prize award-winning precise genome editing technology for gene therapy and crop improvement, Journal of Zhejiang University Science B, 22: 253-284.
https://doi.org/10.1631/jzus.B2100009
PMid:33835761 PMCid:PMC8042526

Lima B., Cappa E., Silva-Junior O., Garcia C., Mansfield S., and Grattapaglia D., 2019, Quantitative genetic parameters for growth and wood properties in *Eucalyptus* “urograndis” hybrid using near-infrared phenotyping and genome-wide SNP-based relationships, PLoS One, 14(6): e0218747.
https://doi.org/10.1371/journal.pone.0218747
PMid:31233563 PMCid:PMC6590816

Liu Q., Yang F., Zhang J., Liu H., Rahman S., Islam S., Ma W., and She M., 2021, Application of CRISPR/Cas9 in crop quality improvement, International Journal of Molecular Sciences, 22(8): 4206.
https://doi.org/10.3390/ijms22084206
PMid:33921600 PMCid:PMC8073294

Min T., Hwarari D., Li D., Movahedi A., and Yang L., 2022, CRISPR-based genome editing and its applications in woody plants, International Journal of Molecular Sciences, 23(17): 10175.
https://doi.org/10.3390/ijms231710175
PMid:36077571 PMCid:PMC9456532

Molla K., Sretenovic S., Bansal K., and Qi Y., 2021, Precise plant genome editing using base editors and prime editors, Nature Plants, 7: 1166-1187.
https://doi.org/10.1038/s41477-021-00991-1
PMid:34518669

Mphahlele M., Isik F., Mostert-O’Neill M., Reynolds S., Hodge G., and Myburg A., 2020, Expected benefits of genomic selection for growth and wood quality traits in *Eucalyptus grandis*, Tree Genetics & Genomes, 16: 49.
https://doi.org/10.1007/s11295-020-01443-1

Myburg A., Grattapaglia D., Tuskan G., Hellsten U., Hayes R., Grimwood J., Jenkins J., Lindquist E., Tice H., Bauer D., Goodstein D., Dubchak I., Poliakov A., Mizrachi E., Kullan A., Hussey S., Pinard D., Merwe K., Singh P., Jaarsveld I., Silva-Junior O., Togawa R., Pappas M., Faria D., Sansaloni C., Petroli C., Yang X., Ranjan P., Tschaplinski T., Ye C., Li T., Sterck L., Vanneste K., Murat F., Soler M., Clemente H., Saidi N., Cassan-Wang H., Dunand C., Hefer C., Bornberg-Bauer E., Kersting A., Vining K., Amarasinghe V., Ranik M., Naithani S., Elser J., Boyd A., Liston A., Spatafora J., Dharmwardhana P., Raja R., Sullivan C., Romanel E., Alves-Ferreira M., Külheim C., Foley W., Carocha V., Paiva J., Kudrna D., Brommonschenkel S., Pasquali G., Byrne M., Rigault P., Tibbits J., Spokevicius A., Jones R., Steane D., Vaillancourt R., Potts B., Joubert F., Barry K., Pappas G., Strauss S., Jaiswal P., Grima-Pettenati J., Salse J., Peer Y., Rokhsar D., and Schmutz J., 2014, The genome of *Eucalyptus grandis*, Nature, 510: 356-362.
https://doi.org/10.1038/nature13308
PMid:24919147

Müller B., Filho J., Lima B., Garcia C., Missiaggia A., Aguiar A., Takahashi E., Kirst M., Gezan S., Silva-Junior O., Neves L., and Grattapaglia D., 2018, Independent and joint-GWAS for growth traits in *Eucalyptus* by assembling genome-wide data for 3 373 individuals across four breeding populations, The New Phytologist, 221(2): 818-833.
https://doi.org/10.1111/nph.15449
PMid:30252143

Paludeto J., Grattapaglia D., Estopa R., and Tambarussi E., 2021, Genomic relationship-based genetic parameters and prospects of genomic selection for growth and wood quality traits in *Eucalyptus benthamii*, Tree Genetics & Genomes, 17: 38.
https://doi.org/10.1007/s11295-021-01516-9

Plasencia A., Soler M., Dupas A., Ladouce N., Silva-Martins G., Martinez Y., Lapierre C., Franche C., Truchet I., and Grima-Pettenati J., 2016, *Eucalyptus* hairy roots, a fast, efficient and versatile tool to explore function and expression of genes involved in wood formation, Plant Biotechnology Journal, 14(6): 1381-1393.
https://doi.org/10.1111/pbi.12502
PMid:26579999

Resende M., Resende M., Sansaloni C., Petroli C., Missiaggia A., Aguiar A., Abad J., Takahashi E., Rosado A., Faria D., Pappas G., Kilian A., and Grattapaglia D., 2012, Genomic selection for growth and wood quality in *Eucalyptus*: capturing the missing heritability and accelerating breeding for complex traits in forest trees, The New Phytologist, 194(1): 116-128.
https://doi.org/10.1111/j.1469-8137.2011.04038.x
PMid:22309312

Sun Y., Jiang C., Jiang R., Wang F., Zhang Z., and Zeng J., 2021, A novel NAC transcription factor from *Eucalyptus*, EgNAC141, positively regulates lignin biosynthesis and increases lignin deposition, Frontiers in Plant Science, 12: 642090.
https://doi.org/10.3389/fpls.2021.642090
PMid:33897732 PMCid:PMC8061705

Thapliyal G., Bhandari M., Vemanna R., Pandey S., Meena R., and Barthwal S., 2022, Engineering traits through CRISPR/Cas genome editing in woody species to improve forest diversity and yield, Critical Reviews in Biotechnology, 43: 884-903.
https://doi.org/10.1080/07388551.2022.2092714
PMid:35968912

Thumma B., Southerton S., Bell J., Owen J., Henery M., and Moran G., 2010, Quantitative trait locus (QTL) analysis of wood quality traits in *Eucalyptus nitens*, Tree Genetics & Genomes, 6: 305-317.
https://doi.org/10.1007/s11295-009-0250-9

Valenzuela C., Ballesta P., Ahmar S., Fiaz S., Heidari P., Maldonado C., and Mora-Poblete F., 2021, Haplotype- and SNP-based GWAS for growth and wood quality traits in *Eucalyptus cladocalyx* trees under arid conditions, Plants, 10(1): 148.
https://doi.org/10.3390/plants10010148
PMid:33450896 PMCid:PMC7828368

Wang J., Matthews M., Williams C., Shi R., Yang C., Tunlaya-Anukit S., Chen H., Li Q., Liu J., Lin C., Naik P., Sun Y., Loziuk P., Yeh T., Kim H., Gjersing E., Shollenberger T., Shuford C., Song J., Miller Z., Huang Y., Edmunds C., Liu B., Sun Y., Lin Y., Li W., Chen H., Peszlen I., Ducoste J., Ralph J., Chang H., Muddiman D., Davis M., Smith C., Isik F., Sederoff R., and Chiang V., 2018, Improving wood properties for wood utilization through multi-omics integration in lignin biosynthesis, Nature Communications, 9: 1579.
https://doi.org/10.1038/s41467-018-03863-z
PMid:29679008 PMCid:PMC5910405

Wang T., Zhang C., Zhang H., and Zhu H., 2021a, CRISPR/Cas9-mediated gene editing revolutionizes the improvement of horticulture food crops, Journal of Agricultural and Food Chemistry, 69(45): 13260-13269.
https://doi.org/10.1021/acs.jafc.1c00104
PMid:33734711

Wang Z., Li L., and Ouyang L., 2021b, Efficient genetic transformation method for *Eucalyptus* genome editing, PLoS One, 16(5): e0252011.
https://doi.org/10.1371/journal.pone.0252011
PMid:34029322 PMCid:PMC8143419

Zhu X., Weng Q., Bush D., Zhou C., Zhao H., Wang P., and Li F., 2023, High-density genetic linkage mapping reveals low stability of QTLs across environments for economic traits in *Eucalyptus*, Frontiers in Plant Science, 13: 1099705.
https://doi.org/10.3389/fpls.2022.1099705
PMid:37082511 PMCid:PMC10112524