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Synergizing Biotechnological Interventions for Resilient Forest Crops

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Biotechnology provides a powerful solution to the steep biological bottlenecks—such as long juvenile phases and high heterozygosity—that have historically stalled conventional forest tree breeding. By leveraging *Agrobacterium*-mediated transformation and CRISPR-Cas9 genome editing, forest enhancement has shifted from slow, generational selection to precise trait engineering.These technologies have successfully optimized trees for commercial value (lowered lignin for easier pulping, enhanced nitrogen use, and increased biomass) while simultaneously building climate and pest resilience (drought, freeze, insect, and blight resistance).

Introduction

Forest trees are critical biological resources providing timber, pulp, bioenergy, carbon sequestration, and diverse ecosystem services. Conventional breeding is severely constrained by long juvenile phases, large genomes, high heterozygosity, and complex reproductive systems. Molecular biotechnology has opened transformative new avenues to overcome these limitations. Two technologies have proven most significant *viz.*, *Agrobacterium*-mediated genetic transformation and CRISPR-Cas9 genome editing. *Agrobacterium tumefaciens* naturally transfers T-DNA into plant genomes; scientists exploit this to stably integrate genes conferring insect resistance, disease tolerance, abiotic stress adaptability, modified wood chemistry, and phytoremediation capacity in species such as poplar, eucalyptus, chestnut, and pine (Yin et al., 2021). CRISPR-Cas9 enables precise editing of endogenous genes using guide RNA-directed nuclease activity without mandatory foreign DNA introduction, with applications spanning wood formation, stress tolerance, growth, flowering, and reproductive biology (Fan et al., 2023; Cao et al., 2024). Notably, CRISPR-mediated disruption of floral and pollen development genes has produced male-sterile lines in eucalyptus and poplar, addressing the critical concern of unintended transgene flow from improved plantations (Elorriaga et al., 2021; Nagle et al., 2023).

Agrobacterium-Mediated Transformation

The *Agrobacterium*-mediated transformatio n is the most applied method in plant genetic modification due to its simplicity and efficiency. Genetically modified trees are those trees whose genomes are being modified by biotechnology tools in order to meet the desired traits.Hybrid poplar transformed with Cry1Ac and Cry3Aa from Bacillus thuringiensis expresses delta-endotoxin proteins that bind larval midgut receptors, inducing osmotic shock and mortality in defoliating insects. This provides a sustainable alternative to chemical insecticides in plantation forestry.American chestnut, nearly eliminated by Cryphonectria parasitica blight, has been restored through Agrobacterium-mediated introduction of wheat oxalate oxidase (OxO), which detoxifies oxalic acid secreted by the pathogen, preventing necrotic canker formation (Powell et al., 2019). Multi-gene transformation of white poplar and silver birch with DREB1A, JERF36, and mtlD upregulates mannitol and proline biosynthesis, stabilising membranes under drought and salinity. Eucalyptus globulus engineered with codA synthesises glycine betaine for cold-stress adaptation, enabling plantation expansion into sub-zero zones Etsuko et al., 2012).RNAi-mediated down-regulation of 4CL and CCoAOMT in aspen and hybrid poplar reduces lignin density and alters the S/G ratio, significantly lowering pulping energy costs . Loblolly pine transformed with bacterial merA, enabling in-situ phytoremediation of mercury-contaminated soils (Doty, 2008).

Table 1 ***Agrobacterium*-Mediated Transformation in Forest Crops**

Species / Tree Crop	Trait Targeted	Key Gene	Donor Organism	Mechanism & Application	Reference
Eucalyptus	Cold & Freezing Tolerance — Plantation Expansion	codA (Choline Oxidase)	Arthrobacter globiformis	Catalyses synthesis of glycine betaine (GB), a compatible osmolyte stabilising cell membranes and enzyme complexes under chilling stress. Enables expansion of commercial plantations into sub-zero temperature zones.	Etsuko et al. (2012)
Loblolly Pine	Phytoremediation — Heavy Metal Detoxification	merA (Mercuric Reductase)	Escherichia coli	Converts highly toxic ionic mercury (Hg^{2+}) into volatile, less toxic elemental mercury vapour (Hg^0) via enzymatic reduction. Enables in-situ extraction and volatilisation of heavy metals from contaminated soils.	Doty (2008)
American Chestnut	Blight Resistance — Cryphonectria parasitica	OxO (Oxalate Oxidase)	Triticum aestivum (wheat)	Wheat OxO detoxifies oxalic acid secreted by the blight fungus, preventing necrotic canker formation and dramatically improving tree survival.	Powell et al. (2019)

CRISPR-Cas9 Genome Editing

This method has been successfully used to boost plant resilience against abiotic stress factors like drought and extreme temperatures. The main advantage of this technology is that there is no external gene that is added to the plant to be modified; it only modifies the plant's own DNA to achieve the intended trait.Editing of SAG12 and WRKY53 extended leaf photosynthetic activity in poplar, increasing biomass and carbon sequestration per rotation (Chen et al., 2022). Lignin pathway edit targeting 4CL and CCR in *P. tomentosa* and MYB and LAC in eucalyptus resulted in reduced lignin content and improved pulp digestibility and biofuel conversion efficiency (Zhou et al., 2015; Chanoca et al., 2019).Preventing transgene flow from improved plantations to wild tree populations is a major regulatory and ecological concern. CRISPR-Cas9 has provided effective biocontainment solutions. Disruption of the LEAFY (LFY) gene in eucalyptus produced abnormal floral development and strongly reduced reproductive capacity, with normal vegetative growth maintained (Elorriaga et al., 2021). Knockout of pollen development genes ETDF1, EREC8, and EHEC3-like generated male-sterile eucalyptus lines with maintained vegetative performance (Nagle et al., 2023). In poplar, PopSAP disruption caused reproductive sterility while preserving desirable growth traits (Azeez & Busov, 2020).CRISPR technology allows for the precise deletion or addition of specific genetic sequences to achieve desired traits. By utilizing the CRISPR/Cas9 system, researchers successfully edited Caffeoyl shikimate esterase (CSE)—a key enzyme in lignin production—in hybrid poplars (Populus alba × P. glandulosa). This targeted modification improves the quality of the trees' biomass, making it easier to convert into biofuels (Jhang et al., 2021).High lignin content provides structural support for plants but hinders industrial wood processing, making low-lignin wood highly desirable for manufacturing. CRISPR-mediated knockout of PagGLR2.8 successfully reduced lignin in modified lines, while simultaneously increasing phloem and xylem fiber lengths. These results demonstrate that editing PagGLR2.8 is an effective approach to optimizing lignin, cellulose, and phloem fiber structures for high-performance composites (An et al., 2025).

Table 2 — CRISPR-Cas9 Genome Editing Applications in Forest Crops

Species / Tree Crop	Trait Targeted	Key Gene	Donor Organism	Mechanism & Application	Reference
Poplar (Populus spp.)	Delayed Senescence — Leaf Longevity / Biomass	SAG12, WRKY53	Endogenous editing — no donor	Extended photosynthetic activity per growing season due to delayed leaf ageing, resulting in significantly increased total biomass production and improved carbon sequestration potential per rotation cycle.	Chen et al. (2022)
Chinese White Poplar (Populus tomentosa)	Lignin & S/G Ratio — Pulp Processing Efficiency	4CL, CCR	Endogenous editing — no donor	Reduction in total lignin content and alteration of S/G ratio leads to improved biomass digestibility and lower energy cost for chemical pulp processing and lignocellulosic bioethanol production.	Zhou et al. (2015)

Eucalyptus (Eucalyptus spp.)	Pulp & Biofuel Digestibility — Cellulose Accessibility	MYB, LAC	Endogenous editing — no donor	Engineered lignin composition enhances enzymatic accessibility of cellulose and hemicellulose, improving paper pulp yield and efficiency of lignocellulosic biofuel conversion in biorefinery applications.	Chanoca et al. (2019)
Eucalyptus (Eucalyptus spp.)	Floral Sterility — Reproductive Containment	LEAFY (LFY)	Endogenous editing — no donor	Targeted LFY gene alteration resulted in abnormal floral development and a substantial reduction in reproductive capacity. Vegetative growth and leaf morphology were largely maintained, enabling effective biological containment.	Elorriaga et al. (2021)
Eucalyptus (Eucalyptus spp.)	Male Sterility — Pollen Development Genes	ETDF1, EREC8, EHEC3-like	Endogenous editing — no donor	Edited lines exhibited severe defects in pollen production and pollen viability, resulting in strong male-sterile phenotypes while maintaining normal vegetative performance in plantation trees.	Nagle et al. (2023)

Conclusion

Future forest resilience requires an integrated tree breeding approach that unifies advanced genetic technologies specifically genome editing, and transgenics This alignment is critical to developing high-performing, climate-resilient genotypes capable of withstanding emerging pathogens and accelerating environmental challenges.

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