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A review on breeding and biotechnological advances for tree farming

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ABSTRACT: *Tree-based farm diversification is vital for climate risk management, resource supply, and farmer livelihoods. Successful agroforestry expansion depends on cultivating economically and ecologically aligned tree species. Long breeding cycles, often spanning several decades, hinder the rapid development of improved tree varieties, making it difficult to respond effectively to changing environmental conditions and market demands. Genetic variation constraints in some tree species limit the range of traits that can be improved through conventional breeding, potentially leaving trees vulnerable to pests, diseases, or changing climates. Additionally, the complex genetics of trees, characterized by polygenic control and genetic interactions, complicate trait selection and breeding decisions. Traditionally, timber and wood product demands were met through natural forest resources, but rapid industrialization and population growth have led to their overexploitation, especially in India. As a result, there is increasing interest in developing improved tree genotypes for integration into farming systems. However, conventional tree breeding is time-consuming, prompting the adoption of biotechnological tools such as Marker-Assisted Selection, GWAS, genome sequencing, genetic engineering, and QTL mapping to accelerate genetic gains and tree improvement. These technologies enhance productivity, product quality, and climate adaptability, supporting the development and widespread adoption of multipurpose agroforestry species to meet diverse economic and ecological needs. Future progress in molecular genetics and genomics is expected to overcome the challenges of breeding trees for integration under farming systems.*

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1. INTRODUCTION

India's forest and tree cover has been steadily increasing from few decades. The total forest cover of the country has increased from 19.53% in the 1980s to 21.71% in 2021 (ISFR, 2021), and its total green cover, including tree cover, now stands at 24.62%.Despite a consistent increase in India's forest and tree cover over the past twenty years, the country still faces a shortfall in timber production. A growing portion of the escalating demand for timber is now being fulfilled through imports. India's wood imports are mostly in the form of roundwood (logs) of which pine and teak are destined for high value wood furniture, and other timber species are used for other purposes including middle segment furniture and construction. In value terms, India's imports of pulp rose from about USD 240 million in 2009 to USD 510 million in 2019, while wood and other wood products increased from USD 1,331 million to about USD 1,950 million over the same period. The furniture and plywood industry is expected to grow considerably as a consequence of the growing middle-class population, increasing urbanization and rising disposable incomes. Specifically, the roundwood demand for plywood industries is anticipated to grow from 17.88 million m^3 RWE (Roundwood Equivalent) in 2022 to approximately 57.49 million m^3 RWE by 2030.This reliance on wood imports uses precious foreign exchange, despite the ability to grow timber species domestically. A major share of the domestic supply of timber can be met from trees cultivated in non-designated forest regions, specifically grown under agroforestry and short rotation forestry (SRF). However, with limited land resources, a major challenge is to upgrade the productivity and resilience of current land use systems.

In India, research and development programmes on promoting agroforestry over the past few decades has been spread over time and regions but, the speedy transition of tree-based farming in the country is still a challenge (Singh *et al*., 2023).The majority of farmers

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in developing countries including India are subsistence farmers, and unable to adopt agroforestry technologies due to economic, infrastructural, policy, and/or socio-cultural issues. Lack of institutional infrastructure, awareness and skill development programmes, insurance, and credit and marketing networks are some reported constraints in adoption of agroforestry (Singh *et al.*, 2023). Additionally, restrictions on tree felling on farmers' land discourage tree-based farming. Scarce quality planting stock, lacking certification systems for agroforestry goods and a dearth of relevant elite genotypes further impede progress (Thakur *et al.*, 2022).

The requirement for timber and products derived from wood in the past was being fulfilled by utilizing resources from natural forests. In recent times, the combination of industrial growth and population increase has resulted in excessive utilization of worldwide forest resources to cater to the need for land and wood-based items. This has brought about a reduction in the availability of natural forest resources in various nations including India. According to ISFR 2023, India's combined forest and tree cover has increased to 827,357 km^2, covering 25.17 % of its geographical area (with forest cover at 715,343 km^2, 21.76 %, and tree cover at 112,014 km^2, 3.41 %) a net gain of 1,445 km^2 since 2021. Although this indicates recovery, it masks ongoing habitat degradation and the uneven domestication efforts across species. As a result, the majority of tree species continue to exist in their natural wild state, while only a handful have begun the process of domestication, undergoing rather limited selection and management (Miller and Gross, 2011).

Integration of multipurpose indigenous tree species within existing farming systems can not only diversify the farm ecosystems but also offer economic stability and livelihood assurance for subsistence farmers while simultaneously addressing environmental concerns through emulation of nature's original state (Leakey *et al.*, 2014). Essentially, this process is guided by farmers and market dynamics, wherein the diverse genetic makeup of economically significant indigenous tree species is harnessed to meet a wide array of product demands in the market (Simons and Leakey, 2004). Therefore, establishing a strong linkages between forest- based industries, farmers, and the research programs is prerequisite for understanding industrial requirements, for better delivery of the end product and for assisting farmers in realizing higher returns (Leakey *et al.*, 2014). This clearly indicates the need to develop the improved tree stocks that matches the need of farmers, industrial needs, and the ecological conditions of the area. This underscores the pressing need to prioritize research for developing genotypes that align with both farmer and industry requirements. However, for developing ideal genotypes for integration on farming system is mostly dependent on the conventional tree breeding that usually takes years to decades of testing and identification of promising genotypes for promotion among farmers (Whetten *et al.*, 2023). With increasing pressure on natural forests, tree farming offers a sustainable alternative that supports afforestation, agroforestry, and reforestation efforts across diverse agro-climatic zones (Singh, 2023). Advances in genomics and biotechnology have significantly enhanced tree breeding and propagation techniques, enabling the development of superior tree varieties with improved growth, wood quality, disease resistance, and stress tolerance (Muller *et al.*, 2019; Yin *et al.*, 2021). Molecular tools such as genome-wide association studies (GWAS), marker-assisted selection (MAS), and genetic engineering accelerate the identification and transfer of desirable traits, thereby reducing breeding cycles and increasing genetic gains (Myburg *et al.*, 2014; Zhou *et al.*, 2020). Biotechnological interventions like micropropagation and somatic embryogenesis facilitate mass clonal multiplication of elite genotypes, ensuring uniformity and high productivity in plantations (Merkle *et al.*, 2014). Overall, integrating these modern approaches with traditional tree farming practices strengthens India's forestry sector and supports sustainable development goals (Thakur *et al.*, 2022). Therefore, biotechnological interventions are need of the hour to accelerate the pace of conventional tree breeding progammes and maximize the genetic improvement (Khlestkina and Shavrukov, 2022). Marker Assisted Selection, Genome-wide association mapping, Genome Sequencing, Genetic engineering, QTL mapping are some of the biotechnological interventions that can revolutionize tree breeding. Table 1 represents a brief overview of conventional and genomic innovations for tree improvement in agroforestry. Now the above-mentioned techniques are being deployed to obtain increased productivity and quality of the trees products and increasing adaptability of the trees to climate change.

However, for developing ideal genotypes for integration on farming system is mostly dependent on the conventional tree breeding that usually takes years to decades of testing and identification of promising genotypes for promotion among farmers (Whetten *et al.*, 2023). Therefore, biotechnological interventions are need of the hour to accelerate the pace of conventional tree breeding progammes and maximize the genetic improvement (Khlestkina and Shavrukov, 2022). Marker Assisted Selection, Genome-wide association mapping, Genome Sequencing, Genetic engineering, QTL mapping are some of the biotechnological interventions that can revolutionize tree breeding. Now the above mentioned techniques are being deployed to obtain increased productivity and quality of the trees products and increasing adoptability of the trees to climate change.

This review article centers on recent progress in fundamental research for developing tree with desirable traits and quality that can be integrated on farms for as per farmer requirements, industrial demands, and ecological conditions. This will ensure mass cultivation and integration of multipurpose agroforestry tree species and revolutionize adoption of industrial agroforestry in the country.

2. BREEDING APPROACHES FOR TREE IMPROVEMENT

Genetically improved trees typically exhibit heightened disease resistance, enhanced durability, and the capacity to yield high-quality seeds. This culminates in progeny that exhibit both phenotypical and genotypical superiority. This achievement owes itself to meticulous plantation efforts, underpinned by precise molecular genetics treatments. This approach serves to alleviate strain on natural forests while concurrently meeting national demands for public benefits. Analogous to the development of improved variants in crops like corn, beans, wheat, vegetables, and fruits, researchers in the field of arboriculture can deliberately select advantageous attributes for trees intended for conservation purposes. A prevalent objective among landowners involves establishing tree plantations populated with robust conservation seedlings endowed with genetic programming conducive to the cultivation of premium timber. For instance, the preference might be for rapid-growth trees to counter vulnerabilities to biotic factors such as animals, parasites, and insect pests. Alternatively, this choice might stem from the desire to abbreviate the rotation period required to attain a commercially viable size, thereby expediting the sale process. The inherent challenges presented by trees, including their large size, prolonged regeneration cycles, and sporadic seed production, pose complexities in the arena of tree improvement endeavors. Nevertheless, breeding and biotechnological approaches provides avenues for resolving these issues and expediting the pace of tree enhancement initiatives.

Tree breeding constitutes a fundamental approach in the field of forest genetics and tree improvement. Tree breeding is traditionally viewed as the conventional method for improving the genetic traits of trees, whereas biotechnology is recognized as a modern, more advanced technological approach to achieving genetic enhancement. Tree improvement encompasses essential procedures such as evaluating and selecting seed sources through provenance trials, scrutinizing and choosing parent trees through progeny testing, and establishing and maintaining seed orchards to yield enhanced seeds and seedlings for reforestation endeavors (White, 1987). The breeding component of tree improvement entails a multi-generational initiative aimed at generating a new cohort of improved trees (comprising selected superior parent trees for enhanced orchards) during each successive breeding cycle. Ideally, the subsequent generation of trees should yield further enhancements or genetic advancements in the specified trait(s) upon being cultivated in the designated environment(s) in comparison to the trees of the preceding generation. The problems encountered in tree breeding are the large size of the genome, scarcity of multigenerational pedigrees and long generation times (Tulsieram *et al*., 1992), this is combined with the non-availability of adequate number of morphological markers in conventional tree breeding.

Additionally, challenges in breeding woody perennials arise from their outcrossing reproductive systems, prolonged juvenile stages, large genome sizes lacking mechanisms to remove long-terminal transposons and an excessive focus on productivity at the expense of adaptive traits (Burdon and Klapste,2019). However, predictive genomics can enhance selection precision and shorten generation intervals. It also addresses the identification of novel variants from tree germplasm (Migicovsky and Myles, 2017) and the revelation of genomic potential for climate adaptation as well (Lind *et al*., 2018). Therefore, predictive genomics ultimately guide conservation and breeding efforts to improve tree health, ecosystem services, and sustainable yield.

3. BIOTECHNOLOGICAL APPROACHES FOR TREE IMPROVEMENTS

Biotechnology presents an array of appealing prospects, including heightened productivity, reduced land pressure, genetic diversity preservation, and improved pest control. Various biotechnologies offer distinct advantages. Typically, genetic gains from traditional breeding programs span 5 to 20% and beyond over generations. Biotechnology is expected to yield atleast comparable, if not faster, benefits than conventional genetics. Achieving this involves propagating valuable genotypes through vegetative means (Mullin and Park, 1992) and enhancing tree growth. This growth enhancement often revolves around manipulating growth-regulating gene synthesis (Von Arnold *et al.*, 1991), seen in tobacco's ethylene biosynthesis enzyme gene (Medford *et al.*, 1989). Another avenue is bolstering tree resilience to environmental stresses. Genes controlling heat tolerance, drought tolerance and low-nutrient tolerance are present in plants. Optimistically, inserting such genes into trees could enhance growth and maybe enable tree planting in poorly managed or degraded sites.

i. In vitro culture

This technique involves cultivating plant tissues, including individual cells, within a sterile and controlled environment. In the last ten years, about 34% of biotechnological activities related to forestry have been dedicated to propagation, as evidenced by Wheeler (2014). Notably, a complete tree can be regenerated from a single cell. Noteworthy instances include the more than eight centuries of clonal forestry through cuttings with Chinese fir (Cunninghamia lanceolata) in China and the clonal propagation of Japanese cedar (Cryptomeria japonica) through cuttings in Japan since the early 15th century (Toda, 1974). The simplicity of propagating tree species through cuttings varies; species with easy rootability, such as poplars (*Populus* spp.), willows (*Salix* spp.), specific *Eucalyptus* species, as well as conifers like spruces (*Larix* spp.), redwood (*Sequoia sempervirens*), and certain pines (*Pinus* spp.), find extensive use in familial or clonal plantations (Menzies and Aimers-Halliday, 2004). While propagation technologies have predominantly served in establishing genetically improved lineages or clones, they also contribute to conserving species of endangered, rare, or vulnerable nature, and those of special cultural, economic, or ecological importance (Benson, 2003).

ii. Genetic engineering

Genetic Engineering (GE) is a powerful technique that employs recombinant DNA and asexual gene transfer methods to modify the genetic structure or regulate the expression of specific genes and traits within organisms, including trees. It offers a transformative approach to introducing and enhancing traits that are challenging to achieve through conventional tree breeding methods, such as distant hybridization and inter-specific hybridization (Fang and Han, 2019). Moreover, GE enables the simultaneous improvement of multiple traits in forest trees, enhancing the efficiency of trait enhancement efforts (Martinez Gomez, 2019).

While the application of GE has yielded promising results in tree species like Populus and Eucalyptus spp., there remains a critical need for the development of efficient genetic transformation protocols for essential tree species (Zhou Y. *et al.*, 2020; Guo *et al.*, 2021). This gap underscores the ongoing challenges and opportunities in harnessing the full potential of GE to advance tree improvement efforts, with the ultimate goal of enhancing the resilience, productivity, and sustainability of forest ecosystems and industries.

Genetic engineering based approaches have been applied to enhance insect resistance in a range of tree species, including Eucalyptus, Populus, Picea, Ulmus, Pinus, and Tsuga spp. (Hammerbacher *et al.*, 2014; Merkle *et al.*, 2014). Additionally, GE techniques have been utilized to confer herbicide resistance against acetylchloroaniline in various forest trees, such as Eucalyptus, hybrids of Populus alba, Piceaabies, Quercus spp., and conifers. Disease-resistant transgenic trees have also been successfully developed in species like Hevea brasiliensis and various Populus spp. (Yin *et al.*, 2021).

Furthermore, GE has been employed in Populus spp. to introduce genes that enhance salt tolerance, cold tolerance, and high-temperature tolerance (Wang *et al.*, 2021). Notably, successful GE initiatives have been undertaken to reduce lignin content by suppressing lignin biosynthesis genes in Populus spp. (Cao *et al.*, 2020). These advancements in genetic engineering represent a promising avenue for improving the traits and resilience of tree species. They address challenges such as pest resistance, abiotic stress tolerance, and lignin content. This, in turn, contributes to the sustainable management of forest ecosystems and the forest-based industry.

iii. RNA Interference

RNA silencing stands as an innovative mechanism for gene regulation that curtails transcript levels through either repressing transcription (Transcriptional gene silencing (TGS)) or instigating a sequence-specific RNA degradation process (Post Transcriptional Gene Silencing (PTGS)/RNA interference (RNAi)) (Agrawal *et al.*, 2003). Recent strides in targeted gene mutagenesis and replacement employing the yeast RAD54 gene (Shaked *et al.*, 2005) or zinc-finger nucleases (Wright *et al.*, 2005) hold the potential to eventually yield effective techniques for engineering null alleles in trees. The effectiveness and stability of RNA interference (RNAi) within perennial species, especially in natural contexts, remain inadequately elucidated. In a study spanning two years, Li *et al.* (2008) assessed 56 distinct poplar RNAi transgenic events within field conditions, indicating that RNAi can be notably efficacious for both functional genomics and biotechnological applications in perennial plants.

4. GENOMICS AND MOLECULAR BREEDING

i. Marker assisted improvement

Genetic markers have become essential tools for comprehending, overseeing, and enhancing both natural and cultivated tree populations. The precision of molecular markers aids in unravelling hybridization and species distinctions. While each marker system has its pros and cons, selecting one hinges on purpose, convenience, and cost. An optimal DNA marker should possess traits like Easy availability, high polymorphism and reproducibility, Co-dominant inheritance and recurrent presence in the genome and environmentally and management practice-neutral.

Recent advancements in molecular biology and biotechnology have facilitated swift genotype characterization and the identification of genetic variations through a diverse array of molecular markers (Campbell *et al*., 2003). These markers encompass isozymes, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), directed amplification of mini-satellite regions (DAMD), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs). The utilization of these markers has been extensively explored in various tree species such as Walnut, Yellow Poplar (Liriodendron tulipifera), Sweet gum (Liquidambar styraciflua), and Eucalyptus species, among others, as integral components of tree improvement initiatives.

Molecular markers offer the potential for early selection in hybridization, resistance to diseases, and wood quality – long-held aspirations of tree breeders. However, a solid grounding in the population genetics of native tree species is essential for the successful execution of genetic conservation programs. In light of this context, we emphasize the significance of pivotal molecular marker systems that can be effectively integrated into tree breeding programs. This entails exploring their applications, strategic implementation, and the existing body of evidence supporting their efficacy.

(a) AFLPS (Amplified Fragment Length Polymorphism): AFLP stands out as an ultra-sensitive method to profile genomic DNA across various organisms. Prior to amplification, DNA is digested using restriction endonucleases like Mse1 and EcoR1. Beyond agriculture, this technique finds application in agronomic trait analysis, diagnostics, pedigree examination, forensics, and universal fingerprinting (Pereira *et al*., 2010). While not as prevalent as other PCR-based markers like RAPD, AFLP analysis has been employed in genetic research on tree species such as Larch (Arcade *et al*., 2000) and Neem (Singh *et al*., 2002).

(b) RAPDS (Random Amplified Polymorphic DNA): RAPD method relies on polymerase chain reaction and is among the most widely used molecular techniques for DNA marker development. RAPDs are simpler and more cost-effective compared to RFLPs, as they don't require prior sequence knowledge or radioactive probes. RAPDs yield DNA profiles of varying complexity based on the primer and template utilized. They have found extensive application in tree genetics and breeding, including Olive, *Morus* sp, Neem, *Eucalyptus* sp, and Larch (Awasthi *et al*., 2004; Bhatt *et al*., 2011). A limitation of the technique is its random generation nature and short primer length, making cross-species transfer challenging. Moreover, RAPDs suffer from poor reliability, reproducibility, and sensitivity to experimental conditions.

(c) SSRs (Microsatellites or Simple Sequence Repeats): SSRs, also known as microsatellites, are brief DNA segments derived from short tandemly repeated sequences, often < 6 base pairs long, such as (GA) n, (AAT) n, and (GT) n. These microsatellites are exceptional genetic markers that combine PCR's rapidity and specificity with heightened information per examined locus. Tree genomes, like Eucalyptus spp.,Olive and rubber tree have been characterized using SSRs (Feng *et al*., 2009). These markers are preferred due to their co-dominant expression, multiallelism, and high Polymorphic Information Content (PIC) value.

(d) Minisatellites or Variable Number Tandem Repeats (VNTRs): Minisatellites, also called VNTRs, are DNA segments spanning roughly 10 to 60 base pairs. Comprising repetitive, often GC-rich, variable repeats, these tandemly arranged variant repeats render minisatellites valuable for scrutinizing DNA turnover mechanisms. Their versatile application spans numerous genetic realms. Minisatellites are connected to chromosomal fragile sites and are positioned near recurring translocation breakpoints. These markers serve diverse purposes in various tree species, including rubber tree fingerprinting and diversity analysis as well as linkage map establishment in pedunculate oak (Barreneche *et al*., 1998).

Many tree species have not undergone the extensive domestication processes observed in field crops like wheat, rice, maize, and edible legumes. Hardwood trees exhibit significant diversity in wood anatomy, leaf morphology, overall tree architecture, secondary metabolism, and various adaptive characteristics (Groover and Crook, 2017). Given that the genetic basis of these traits is now well understood, both in model systems and/or cultivated crops and trees, there is an opportunity to accelerate improvement efforts using modern methodologies like genomics. Approaches such as genomics-assisted breeding, quantitative trait locus (QTL) mapping, genome-wide association studies (GWAS), and genomic selection (GS) can streamline enhancement endeavors (Hickey *et al*., 2017). Several studies in tree species such as eucalyptus, poplar, pine, spruce, apple, peach, oil palm, citrus, cocoa, grape, and macadamia have leveraged genomics-based technologies to establish connections or associations between economically significant traits and single nucleotide polymorphism (SNP) variants.

ii. Genome maps

Genome maps have been successfully developed in numerous tree species, with particular emphasis on conifers (Neale and Sederoff, 1996). The initial linkage assessment for a conifer, loblolly pine (*Pinus taeda*), was accomplished using isozymes as genetic markers in haploid megagametophytes (Conkle, 1981). These isozyme-based genetic maps, due to their limited number of loci, covered only a fraction of the linkage groups within the complete genome. Despite this, isozymes continue to play a role in recent genetic mapping efforts in pine species (Goncharenko *et al*., 1998), often in conjunction with DNA markers for comprehensive genome mapping (Sewell *et al*., 1999). Subsequently, with the acquisition of cDNA and genomic probes to ascertain the inheritance of restriction fragment length polymorphisms (RFLPs) in Pinus taeda (Devey *et al*., 1991), the pioneer RFLP-based genetic map was constructed for loblolly pine (Pinus taeda) by Devey *et al*. (1994). This milestone paved the way for the creation of genome maps, including those delineating quantitative trait loci (QTLs), in various other tree species.

iii. QTL and Genome wide association mapping of economic traits

Genetic maps play a pivotal role in pinpointing the locations of both singular gene and polygenic traits within linkage groups, rendering them indispensable for pragmatic breeding endeavors. To exemplify, the dominant major gene responsible for white pine blister rust resistance has been genetically situated within the genome of sugar pine (Pinus lambertiana) (Harkin *et al*., 1998). Polygene mapping (Sewell and Neale, 2000) has achieved successful outcomes for quantitative trait loci (QTLs) associated with traits like wood specific gravity, annual height and diameter increment growth in the diverse progeny of Pinus taeda (Kaya *et al*., 1999). Similarly, QTLs governing adaptive attributes such as timing of vegetative bud flush and spring and fall cold hardiness in *Pseudotsuga menziesii* have been mapped (Jermstad *et al*., 2000), as have QTLs linked to wood strength in *Cryptomeria japonica* (Kuramoto *et al*., 2000). The genetic mapping landscape extends to traits like vegetative propagation traits in *Eucalyptus grandis*, *E. urophylla,* and *E. tereticornis* (Marques *et al*., 1999), and substantial QTLs impacting growth and form in *Populus trichocarpa* and *P. deltoides* (Bradshaw and Stettler, 1995). In a more recent context, Kancharla *et al*. (2019) identified three minor QTLs associated with Jatropha Mosaic Virus (JMV) resistance within an F2 population from crosses between resistant and susceptible individuals, deploying 207 polymorphic SSR markers.

The era of plant genomics has ushered in the development of fundamental tools for genomics-assisted breeding, specifically genome-wide association mapping (GWAS) and genomic selection (GS), powered by sequencing technologies (Bhat *et al*., 2016). This epoch has yielded significant breakthroughs in advancing plant breeding and enhancing the arsenal for crop improvement, particularly in major field crops. Numerous genome-wide association studies continue to unfold across diverse phenotypic spectra, and endeavors to estimate somatic mutation rates within old-growth tree species are currently underway. High-throughput markers, including SNPs and InDels, have been developed using a targeted capture strategy combined with deep sequencing of 94 genes essential for wood formation across three *Eucalyptus* species. This advancement has significantly supported QTL and association studies (Dasgupta *et al*., 2015). Notably, the EuCHIP60K has emerged as a pivotal resource, enabling exploration into genome-wide recombination, linkage disequilibrium, nucleotide diversity, facilitating genome-wide association (Muller *et al*., 2019), and steering genomic selection in various Eucalyptus species (Tan *et al*., 2018). Furthermore, the application of genome-wide association studies and genomic prediction in relation to fruit quality traits has been undertaken in the realm of citrus (Minamikawa, 2017).

iv. Consensus maps and synteny

Genetic information within an individual map can be maximized by constructing consensus genome maps for a species. Such consensus genome maps, amalgamating RFLPs, RAPDs, and isozymes, have been established in Pinus taeda (Sewell *et al*., 1999) by integrating linkage data from two unrelated three-generation outbred pedigrees. The prevailing trend is to harmonize both cytogenetic and molecular genome maps, yielding integrated genetic maps in plants, including trees.

Another facet of genetic mapping involves identifying conserved gene arrangements (synteny) within

Table 1: Overview of conventional and genomic innovations for tee improvement in agroforestry

S.No.	Aspect	Technique/Advance	Examples/Species	References
1.	Conventional Breeding	Selection and Hybridization	Eucalyptus spp., Populus spp., Teak (*Tectona grandis*)	Miller and Gross, 2011; Whetten *et al.*, 2023
2.	Clonal Propagation	Cuttings, Grafting, Micropropagation	Eucalyptus, Casuarina, Populus	Leakey *et al.*, 2014
3.	Marker-Assisted Selection (MAS)	Use of molecular markers linked to traits	Teak (RAPD), Pine (SSR), Eucalyptus (SNP)	Khlestkina and Shavrukov, 2022; Vaishnav *et al.*, 2006
4.	Quantitative Trait Loci (QTL) Mapping	Identifying genome regions linked to complex traits	*Pseudotsuga menziesii* (bud flush timing), Eucalyptus spp.	Jermstad *et al.*, 2000; Kshirsagar *et al.*, 2017
5.	Genome-Wide Association Studies (GWAS)	Association of traits with genetic variants across genome	Populus, Eucalyptus	Khlestkina and Shavrukov, 2022; Kumar *et al.*, 2020
6.	Whole Genome Sequencing	Complete sequencing of tree genomes	*Populus trichocarpa,* *Eucalyptus grandis*	Jiao *et al.*, 2017; Myburg *et al.*, 2014
7.	Transcriptomics	RNA sequencing to study gene expression	Populus under drought stress	Sundell *et al.*, 2015
8.	Genetic Engineering	Direct modification of genes	Transgenic Poplar, Eucalyptus	Kalluri and DiFazio, 2009
9.	CRISPR/Cas9 Genome Editing	Precise gene editing technique	Experimental in Populus and Pine	Zhou *et al.*, 2020
10.	Pangenomics	Study of full genetic diversity across multiple genomes	Emerging in Eucalyptus, Populus	Bayer *et al.*, 2020
11.	Bioinformatics and Machine Learning	Data analysis and predictive modeling	Applied in Populus, Eucalyptus	Varshney *et al.*, 2021

genomes of related species, enabling comparative mapping (McCouch, 2001). These comparative genetic maps exhibit striking colinearity, offering insights into genome structure and evolution within the investigated species (Paterson *et al*., 2000). To ascertain the extent of cross-hybridization across conifers, mapped cDNA and genomic probes from loblolly pine (Pinus taeda) were hybridized with Southern blots containing genomic DNA from various conifer species, including different Pinus species and representatives from genera like *Abies, Picea, Pseudotsuga, Larix, Sequoia, Calocedrus, Torreya*, as well as an angiosperm, *Populus* (Ahuja *et al*., 1994). These investigations revealed that mapped cDNA probes from loblolly pine exhibited cross-hybridization with genomic DNA of other Pinus species and certain genera within Pinaceae. However, only a minor fraction of loblolly pine probes demonstrated hybridization to genomic DNA from three other conifer families (Taxodiaceae, Taxaceae, and Cupressaceae) and the examined angiosperm. This exploration demonstrated the feasibility of comparative genome mapping within pines, and even the broader Pinaceae family, employing a shared set of DNA probes. The practice of comparative mapping, facilitated by shared DNA probes, has been meticulously documented in studies involving Pinus taeda and Pinus radiata (Devey *et al*., 1999). Furthermore, the application of cDNA sequencing and expressed sequence tags (ESTs) has gained prominence as a pivotal approach for genomic analysis.

6. CONCLUSION

Tree-based farm diversification is essential for mitigating climate risks while ensuring sustainable supplies of timber, non-timber products, food security, and livelihoods for subsistence farmers. Effective scaling of agroforestry depends on cultivating improved tree species that balance economic and ecological needs, addressing both farmer requirements and industry demands. Conventional breeding remains slow due to trees' long generation cycles and extended vegetative phases, often spanning decades. Recent advances in tree improvement increasingly utilize sophisticated molecular and genomic tools such as Marker-Assisted Selection, QTL mapping, Genetic Engineering, Genome-Wide Association Studies (GWAS), and cutting-edge tree genomics techniques including whole-genome sequencing, pangenomics, and transcriptomics. For example, whole-genome sequencing of major species like Eucalyptus and Populus has revealed extensive genetic variation, enabling identification of key genes linked to growth, wood quality, and stress tolerance. These technologies accelerate breeding cycles by precisely characterizing genetic diversity and generating novel variation, contributing to higher genetic gains. Future prospects include integrating CRISPR-based genome editing to develop climate-resilient and pest-resistant genotypes, leveraging machine learning for predictive breeding, and expanding pan genomic resources to capture species wide genetic diversity. This genomics-driven approach enhances productivity, shortens rotation periods, improves tolerance to biotic and abiotic stresses, and boosts adaptability to climate change. Ultimately, it facilitates the development of tree genotypes tailored for diverse agroecological zones and market needs, promoting widespread adoption of industrial agroforestry and seamless integration of economically valuable tree species into sustainable farming systems.

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