



## Unlocking the Secrets of Biodiversity in Agroforestry: The Critical Role of Biotechnology for Long-Term Conservation

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### Abstract

Agroforestry systems play a critical role in biodiversity conservation, climate resilience, and sustainable rural livelihoods; however, their ecological integrity is increasingly threatened by land-use intensification, habitat degradation, and unsustainable resource extraction, particularly in drought prone regions such as Bundelkhand in India. This review critically examines the constraints limiting effective agroforestry optimization and evaluates the role of plant biotechnology in strengthening conservation and propagation strategies for priority species. Emphasis is placed on *in vitro* techniques, including micropropagation, synthetic seed technology, somatic embryogenesis, and germplasm conservation, as tools to overcome limitations associated with poor natural regeneration, low seed viability, and overexploitation. This review synthesizes tissue culture advancements and research gaps across key agroforestry species such as *Terminalia arjuna*, *Diospyros melanoxylon*, *Manilkara hexandra*, *Madhuca longifolia*, *Aegle marmelos*, and *Buchanania lanzan*. The integration of species-specific biotechnological protocols with *in situ* conservation and sustainable management frameworks is identified as essential for enhancing genetic resource conservation, large-scale propagation of elite genotypes, and long-term agroforestry system resilience.

**Keywords:** Biodiversity; Natural Resources; Agroforestry

### Introduction

As global agriculture confronts the twin challenges of climate change and declining natural resources, agroforestry has emerged as a powerful model for sustainable land use, one that harmonizes productivity with ecological integrity. However, like biodiversity at large, owing to many unmanageable factors, the biodiversity in agroforestry too is facing an unprecedented crisis. The alarming decline of agroforestry threatens not only the delicate balance of agri-ecosystems but also the very foundations of human well-being, livelihoods, and food security, triggering far-reaching social, environmental, and economic devastation worldwide [19]. This is

the fundamental reason that in an era marked by climate change and increasing pressure on natural resources, the management and augmentation of agroforestry systems within agricultural landscapes has become both a scientific necessity and a societal responsibility.

Increasing population and shrinking land holdings have had a direct impact on agroforestry, which once had an interdependent relationship with agriculture. Agroforestry, once an integral part of agriculture, is now largely neglected due to the increasing trend towards cash crop cultivation. Over time, the tradition of

combining agroforestry with farming became extinct. Failure to integrate agroforestry with farming results in short-term economic inefficiencies and long-term ecological and socio-economic degradation. The widespread ignorance of agroforestry-led dearth of trees results in poor microclimatic regulation, exposing crops to heat stress, strong winds, and moisture loss. Soil fertility declines rapidly because there is no continuous organic matter input from leaf litter or root turnover, increasing dependence on chemical fertilizers and raising cultivation costs. Additionally, farms lose opportunities for on-farm resources such as fuel wood, fodder, fruits, timber, and medicinal products that agroforestry systems can supply even within a few years.

Over the long run, the consequences are far more severe and often irreversible. Continuous cropping without tree integration accelerates soil degradation, including erosion, nutrient depletion, reduced soil organic carbon, and declining biological activity. Water resources are adversely affected due to poor infiltration, increased runoff, and reduced groundwater recharge. The absence of trees also contributes to loss of biodiversity, eliminating habitats for beneficial insects, birds, and microorganisms that naturally regulate pests and diseases. Climate resilience is significantly weakened, as farms without trees have lower carbon sequestration potential and reduced capacity to buffer against climate extremes such as droughts, floods, and temperature fluctuations. Economically, farmers remain trapped in low-resilience, input-intensive systems, facing unstable incomes and limited livelihood security over generations.

### **From promise to practice: Challenges limiting the optimization of agroforestry systems**

Despite the recognized benefits of agroforestry, their transition from conceptual promise to widespread practical implementation remains constrained by multiple interconnected challenges. One of the primary constraints lies in ecological and biophysical complexities. Agroforestry systems require careful species selection, spatial design, and long-term management to balance competition and complementarities between trees and crops. Inadequate understandings of tree - crop interactions, inappropriate species combinations, and site-specific variability in soil, climate, and hydrology often lead to suboptimal productivity [14]. Additionally, long gestation periods of trees discourage farmers seeking short-term returns. Socio-economic barriers further restrict adoption.

The delayed economic returns from timber or fruit trees create financial uncertainty in front of small and marginal farmers. In many regions, unclear land tenure and tree ownership rights discourage farmers from investing in long-term tree cultivation. On the technical front, insufficient extension services, limited farmer training, and lack of context-specific research slow the optimization of agroforestry practices. Institutional and policy-related constraints compound these issues. Complex regulatory frameworks governing tree felling, transit permits, and marketing of forest produce create bureaucratic hurdles.

### **The biotechnological imperative: Addressing agroforestry challenges through innovation**

Biotechnology emerges as a critical tool to address the multifaceted challenges facing agroforestry species conservation. Modern biotechnological approaches, particularly plant tissue culture and micropropagation, offer solutions to overcome the propagation barriers that have long constrained the sustainable management of important agroforestry species. By enabling rapid multiplication of elite genotypes, conservation of endangered germplasm, and production of disease-free planting material, biotechnology bridges the gap between conservation needs and practical implementation. The following sections examine key agroforestry species of the Bundelkhand region and their current status in tissue culture research, highlighting both achievements and opportunities for further development.

### **Priority agroforestry species: Conservation status and tissue culture advances**

#### **Arjun (*Terminalia arjuna* Roxb.)**

Renowned for its medicinal value, ecological functions, and livelihood potential, Arjun holds a distinguished position in traditional Indian medicine, particularly in Ayurveda, where it is regarded as a cardiotonic tree. The bark of the tree is rich in bioactive compounds such as tannins, flavonoids, and glycosides, which contribute to its therapeutic properties [8]. Arjun bark is commonly used to strengthen the heart muscles, regulate blood pressure, improve circulation, and reduce cholesterol levels. Arjun used to be found in abundance along the banks of water sources and lakes in Bundelkhand but its populations are under increasing pressure due to indiscriminate and unscientific bark harvesting, habitat degradation, and poor natural regeneration. Excessive

debarking often leads to tree mortality, while slow growth and limited seed viability hinder population recovery.

Significant advances have been made in the *in vitro* propagation of *Terminalia arjuna*. Researchers have successfully established micropropagation protocols using nodal segment explants from mature plants, with optimal shoot multiplication achieved on modified Murashige and Skoog (MS) medium supplemented with 0.5 mg/l benzylaminopurine (BAP) and 0.1 mg/l naphthalene acetic acid (NAA), producing  $16.50 \pm 3.67$  shoots per explants [6]. Seasonal variations significantly affect proliferation potential, with explants collected during April-May showing the best response [10]. Importantly, synthetic seed production has been developed using shoot tip encapsulation in 3% sodium alginate and 100 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , offering potential for short-term storage and germplasm exchange [10,27]. Despite these advances, genotypic variations in bud break response (ranging from 86.66% to 100%) indicate the need for genotype-specific protocol optimization. Key challenges that have been addressed include culture contamination, phenolic exudation, bud growth inhibition, and shoot yellowing through pre-treatment strategies such as mother tree lopping six months prior to explant collection and media modifications.

#### **Tendu (*Diospyros melanoxylon* Roxb.)**

Also known as the Beedi leaf tree, Tendu is one of the most important non-timber forest produce (NTFP) species of central India, particularly in regions such as Bundelkhand. In this region, Tendu leaf collection serves as a critical source of cash income during lean agricultural periods. Despite its importance, Tendu faces increasing threats due to overexploitation, unsustainable harvesting practices, habitat degradation, grazing pressure, and climate-induced stress. Repeated lopping for leaf collection often weakens trees, reduces regeneration potential, and compromises long-term productivity. Natural regeneration is slow and erratic, further aggravated by declining forest quality and land-use changes.

While comprehensive micropropagation protocols for *Diospyros melanoxylon* remain limited compared to other *Diospyros* species, research on related species such as *D. kaki* and *D. virginiana* provides valuable insights. Studies on *D. virginiana* have demonstrated successful *in vitro* establishment using MS medium with zeatin [18] while *D. kaki* protocols have utilized BAP with auxins for shoot multiplication [9]. Recent recognition of biotechnological

interventions, including tissue culture techniques, has been identified as essential for mass propagation and enhancing resilience of Tendu trees in changing environmental conditions. However, species-specific protocols for *D. melanoxylon* require urgent development, particularly addressing challenges such as phenolic exudation common in this genus and optimizing explant sources and growth regulator combinations for this economically critical NTFP species.

#### **Khirni (*Manilkara hexandra* Roxb.)**

Increasingly popular in India, particularly in dry and semi-arid regions like Bundelkhand, Khirni is valued for its flavor and superfood nutritional profile. The tree is also culturally valued for its longevity and drought tolerance, making it a trusted species in traditional farming systems of water-scarce regions like Bundelkhand. Although largely underutilized at the commercial scale, its fruits have significant potential for value addition in the form of processed products such as pulp, beverages, and traditional sweets. However, the plant's naturally wild and unruly growth habit has made it impractical for large-scale commercial farming [5]. This challenge has kept the highly nutritious crop of Bundelkhand region trapped between its wild origins and its commercial potential, even as global demand continues to rise.

Tissue culture research for *Manilkara hexandra* remains predominantly at the academic and research stage, with limited commercial application. Studies on the related species *Manilkara zapota* (sapodilla) indicate that micropropagation in this genus faces significant challenges due to high phenolic content and latex exudation from explants, which hamper culture establishment and growth [13]. Despite these difficulties, *M. zapota* has been successfully micropropagated using meristem culture on Woody Plant Medium (WPM) with varying BAP concentrations (2-5 ppm), achieving shoot and leaf induction [28]. Notably, *M. hexandra* is commonly used as a rootstock for sapodilla grafting due to its high graft compatibility, demonstrating its propagation potential. However, specialized protocols addressing the unique challenges of *M. hexandra* including management of phenolic compounds, optimization of surface sterilization procedures, and development of suitable rooting protocols are urgently needed for commercial-scale propagation. Given its classification as critically endangered with high risk of extinction, development of efficient tissue culture protocols could play a vital role in both conservation and commercialization of this valuable species.

### Jimaria or Khatua (*Gardenia gummifera* L.f.)

An important indigenous NTFP species of dry deciduous forests and scrublands of central India, including the Bundelkhand region, Jimaria is best known for the resin (gum) exuded from its bark, commonly referred to as Dikamali gum which has been used for centuries in Ayurvedic and local medicinal practices meant for treating inflammatory conditions, joint pain, skin diseases, wounds, and digestive disorders. Its ability to survive under moisture stress enhances its potential as a low-input species that can contribute to livelihood diversification in water-scarce Bundelkhand region without competing heavily with agricultural crops. Like other NTFP species, Jimaria is also facing a gradual decline due to overexploitation of gum resin, acutely poor seed viability, destructive harvesting-grazing practices, and habitat degradation.

Published tissue culture research specifically for *Gardenia gummifera* remains extremely limited. While other *Gardenia* species have been successfully micropropagated using standard protocols involving shoot tip or nodal explants on MS medium with cytokinins [2,23], species-specific protocols for *G. gummifera* are not well documented. Given the species poor seed viability and increasing conservation concerns, development of efficient micropropagation protocols represents a critical research gap. Potential approaches could include optimization of explant sources (shoot tips, nodal segments from juvenile material), systematic screening of cytokinin types and concentrations (BAP, kinetin, TDZ), management of potential phenolic exudation issues, and development of effective rooting and acclimatization procedures. Establishment of tissue culture protocols would be particularly valuable for rapid multiplication of superior genotypes with high gum yield and for conservation of germplasm from threatened wild populations.

### Kaitha (*Feronia limonia* (L.) Swingle)

Also known as Wood Apple, Kathbel, or Elephant Apple in different regions, Kaitha is a hardy indigenous fruit tree widely found in Bundelkhand region of India and remains a trusted tree for its digestive, carminative, and liver-protective properties. It is commonly used in the treatment of diarrhea, dysentery, and stomach disorders. The bark, leaves, and roots are also used in traditional remedies for wounds, skin ailments, and fever. Economically, Kaitha serves as a low-risk perennial income source through the sale of fruits and processed products such as jams,

jellies, and squashes in local and regional markets in drought-prone regions such as Bundelkhand. The species exhibits slow growth and irregular seed germination, which limits large-scale plantation and natural recovery.

Limited published research exists on *in vitro* propagation of *Feronia limonia* [4,11]. As a member of the Rutaceae family, protocols developed for related citrus species may provide useful starting points, though species-specific optimization would be essential. Key research priorities include development of surface sterilization protocols to minimize contamination while maintaining explant viability, optimization of growth regulator combinations for shoot multiplication, establishment of efficient rooting systems, and development of hardening protocols for *ex vitro* transfer. In view of its economic potential and propagation challenges through conventional methods, establishment of reliable tissue culture protocols could significantly enhance availability of quality planting material and support both conservation and commercial cultivation initiatives.

### Lasoda (*Cordia dichotoma* G. Forst.)

Also known as *Cordia myxa* or Gunda, Lasoda occupies a significant place in rural diets and culinary practices in Bundelkhand region. Its mucilaginous pulp is believed to have cooling and soothing properties and is traditionally used to manage digestive disorders, throat irritation, and heat-related ailments. In folk medicine, the fruit pulp, bark, and leaves are used for treating cough, fever, wounds, skin infections, and inflammatory conditions. The growing interest in indigenous and underutilized fruits has further increased the commercial potential of Lasoda. Natural regeneration is often poor, and lack of improved planting material limits its wider adoption in agroforestry and orchard systems.

Tissue culture protocols for *Cordia dichotoma* are not extensively documented in scientific literature. However, the genus *Cordia* has shown amenability to *in vitro* culture in related species [15]. Priority research areas should include identification of suitable explant sources with low contamination rates, development of culture establishment protocols addressing potential oxidative browning, optimization of cytokinin-auxin ratios for shoot proliferation, and establishment of reliable rooting and acclimatization procedures. Development of efficient micropropagation systems would support both germplasm conservation and commercial nursery production in view of its increasing commercial interest and poor natural regeneration.

### Passion fruit (*Passiflora edulis* Sims)

Valued in local diets for its refreshing flavor and health-promoting properties, passion fruit has been widely adopted into farming systems and homestead gardens where it can be grown as a climber on trees, fences, or live supports, thereby optimizing land use and enhancing farm income. Despite its economic potential, passion fruit faces challenges related to genetic erosion, pest and disease susceptibility, and inconsistent availability of quality planting material. Unregulated propagation, dependence on limited cultivars, and lack of systematic conservation efforts threaten the long-term sustainability of the crop.

*Passiflora edulis* has been extensively studied for *in vitro* propagation with well-established protocols available. Micropropagation typically uses nodal or shoot tip explants cultured on MS medium supplemented with BAP or other cytokinins [16]. The species responds well to tissue culture with relatively high multiplication rates [17]. Somatic embryogenesis protocols have also been developed for some cultivars. Key applications include production of virus-free plants through meristem culture, rapid multiplication of superior selections with desirable fruit quality, and conservation of genetic diversity. Despite these advances, challenges remain in maintaining genetic stability over multiple subcultures and optimizing protocols for diverse cultivars. Continued refinement of protocols, particularly for region-specific cultivars and disease resistance, would enhance the contribution of tissue culture to sustainable passion fruit production.

### Star fruit (*Averrhoa carambola* L.)

Commonly known as Carambola, star fruit is cultivated in many parts of India including Bundelkhand region and Southeast Asia and is increasingly being integrated into home gardens, orchards, and agroforestry systems due to its adaptability, health benefits, and growing market demand. In traditional medicine systems, star fruit is used to manage fever, diarrhea, skin ailments, and digestive disorders. Owing to its regular bearing habit, good market price, and potential for value addition, it provides farmers with a profitable enterprise. Unplanned expansion, reliance on a narrow genetic base, challenges related to genetic erosion, limited varietal diversity, vulnerability to pests and diseases, climatic challenges, and inadequate availability of quality planting material threaten long-term productivity and sustainability of star fruit.

In some regions, traditional varieties are being replaced by a few commercial types, leading to loss of genetic diversity.

*In vitro* propagation protocols for *Averrhoa carambola* have been established, though not as extensively as some other tropical fruits. Successful protocols typically employ shoot tip or nodal explants on MS medium with cytokinins (primarily BAP) for shoot multiplication [21]. Challenges include genotype-dependent responses, potential for somaclonal variation, and the need for efficient rooting protocols. Applications include rapid multiplication of elite cultivars with superior fruit quality, conservation of traditional varieties threatened by genetic erosion, and production of disease-free planting material. Further research focusing on protocol refinement for diverse cultivars, reduction of somaclonal variation, and development of cost-effective production systems would enhance the practical utility of tissue culture for star fruit improvement and conservation programs.

### Mahua (*Madhuca longifolia* J.F. Macbr.)

Central to the socio-cultural fabric of rural and tribal societies of central and northern India, particularly in regions such as Bundelkhand, Mahua is deeply embedded in traditional life, food systems, and livelihoods. Often referred to as the tree of life due to its multifaceted utility and resilience in dry and marginal landscapes, the tree provides shade, supports biodiversity, and is traditionally protected by communities due to its importance in daily life. Despite its immense importance, Mahua populations are under increasing threat due to deforestation, land-use changes, overexploitation, and declining natural regeneration. Unsustainable harvesting of flowers and seeds, coupled with grazing pressure and lack of systematic plantation efforts, has adversely affected population structure and productivity.

Micropropagation research on *Madhuca longifolia* has been conducted, though protocols are not yet fully optimized for commercial-scale application. Studies have explored various explant sources including shoot tips and nodal segments, with MS medium supplemented with different cytokinins showing varying degrees of success [22,24,26]. Challenges specific to this species include slow growth rates *in vitro*, potential phenolic exudation, difficulty in rooting, and need for extended acclimatization periods. Priority areas for further research include optimization of growth regulator combinations for improved shoot multiplication

rates, development of effective rooting protocols, establishment of efficient hardening procedures, and evaluation of long-term genetic stability. Given its cultural and economic importance and increasing conservation concerns, refinement of tissue culture protocols would significantly contribute to germplasm conservation and restoration programs.

### **Bel (*Aegle marmelos* (L.) Corrêa)**

Also known as Bael, Bilva, or Sriphal, Bel occupies a unique position where traditional knowledge, religious faith, and economic utility converge. Its multipurpose nature makes it an indispensable component of agroforestry systems and rural livelihoods. The ripe fruit is widely used to treat digestive disorders, chronic diarrhea, dysentery, and heat-related ailments, while unripe fruit is valued for its astringent properties. Leaves and roots are used in formulations for fever, diabetes, inflammation, and respiratory problems. Despite its sacred status and wide utility, Bel is facing gradual decline in natural populations due to habitat loss, land-use changes, neglect of traditional orchards, and poor regeneration in the wild. Overharvesting of fruits and plant parts coupled with lack of systematic cultivation and quality planting materials poses a threat to its long-term sustainability.

*In vitro* propagation protocols for *Aegle marmelos* have been developed with varying degrees of success. Research has utilized multiple explant sources including shoot tips, nodal segments, and cotyledonary nodes [1,3,20]. Successful shoot multiplication has been achieved on MS medium with combinations of BAP and other cytokinins. The species presents specific challenges including contamination issues due to endophytic organisms, oxidative browning of explants, and difficulties in achieving high-frequency rooting [1,7]. Despite these challenges, tissue culture offers valuable tools for conservation of elite genotypes, rapid multiplication of improved cultivars with superior fruit quality, and production of pathogen-free planting material. Further optimization focusing on improved contamination control, enhanced rooting efficiency, and development of cost-effective protocols would strengthen the application of tissue culture in Bel conservation and commercial production programs.

### **Chiraunji (*Buchanania lanzan* Spreng.)**

Also known as Charoli or Achar, Chiraunji is a highly valued indigenous NTFP species of dry deciduous forests of central and

northern India, including the Bundelkhand region. Renowned for its nutritious seeds and cultural relevance, Chiraunji plays an important role in Indian cuisine, especially in festive and ceremonial foods, indigenous medicine, and forest-based livelihoods. Poor natural regeneration, habitat loss, overexploitation, and destructive harvesting methods are the key factors driving declining Chiraunji populations. The species exhibits slow growth, irregular flowering, and low seed germination, which severely limits its natural recovery. Increased commercial demand has intensified pressure on wild populations, threatening long-term sustainability.

Published tissue culture research on *Buchanania lanzan* is limited, reflecting the species status as an underutilized crop, however reliable protocols have been established using embryo rescue technique [12], using young leaf and nodal segments [25]. This represents a significant research gap given the species economic importance and conservation needs. Development of efficient micropropagation protocols would require systematic investigation of explant sources (preferably from juvenile material to maximize regeneration potential), optimization of surface sterilization procedures, screening of cytokinin types and concentrations, evaluation of auxins for rooting, and development of effective acclimatization procedures. Specific challenges may include slow growth rates characteristic of the species, potential phenolic exudation issues, and need for specialized rooting conditions. Establishment of reliable tissue culture systems would be particularly valuable for conservation of germplasm from threatened wild populations and for rapid multiplication of superior genotypes identified for high seed yield and quality.

### **Conclusion**

The integration of biotechnological approaches, particularly plant tissue culture and micropropagation, represents a critical pathway for addressing the multifaceted challenges facing agroforestry species conservation in the Bundelkhand region and beyond. While significant progress has been achieved for some species such as *Terminalia arjuna* and *Passiflora edulis*, substantial research gaps remain for many economically and ecologically important species including *Diospyros melanoxyton*, *Manilkara hexandra*, *Gardenia gummifera*, and *Buchanania lanzan*.

The successful application of tissue culture technology requires species-specific protocol optimization, addressing challenges

such as phenolic exudation, genotype-dependent responses, contamination control, and efficient rooting and acclimatization procedures. Beyond technical aspects, the translation of laboratory protocols to commercial-scale production demands attention to cost-effectiveness, infrastructure development, and capacity building among local stakeholders. Furthermore, tissue culture efforts must be complemented by *in situ* conservation strategies, habitat restoration, sustainable harvesting practices, and supportive policy frameworks to ensure long-term success.

As climate change intensifies and pressure on natural resources grows, the imperative for biotechnological intervention in agroforestry conservation becomes increasingly urgent. Investment in research and development of tissue culture protocols, coupled with their integration into broader conservation and livelihood enhancement programs, will be essential for unlocking the full potential of agroforestry systems in supporting biodiversity, ecosystem services, and sustainable rural development. The path forward requires collaborative efforts among researchers, policymakers, conservation practitioners, and local communities to harness biotechnology as a powerful tool in the quest for long-term agroforestry biodiversity conservation.

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