



Tissue culture in mulberry (*Morus spp.*): Techniques, applications, challenges and future prospects

G Savitha

Research scholar, Department of Sericulture, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Abstract

Sericulture is a vital agro-based industry that depends largely on the quality and productivity of mulberry (*Morus spp.*), the exclusive host plant of the silkworm (*Bombyx mori* L.). Improvement of mulberry for enhanced leaf yield, nutritional quality, and stress tolerance is therefore essential for sustainable silk production. However, conventional breeding approaches are time-consuming and constrained by the perennial nature, high heterozygosity, and complex genetics of mulberry. In this context, plant tissue culture has emerged as an efficient biotechnological approach for rapid multiplication, genetic conservation, and crop improvement. This review presents a comprehensive overview of tissue culture techniques in mulberry, including micropropagation, callus culture, somatic embryogenesis, and organogenesis, along with their role in large-scale propagation and production of disease-free planting material. Applications such as synthetic seed technology, cryopreservation, and *in vitro* screening for stress tolerance are also discussed. Furthermore, the integration of tissue culture with modern molecular tools such as marker-assisted selection, genetic transformation, and CRISPR/Cas9 genome editing offers new opportunities for precise genetic improvement of mulberry. Despite these advances, challenges including somaclonal variation, contamination, high production costs, and acclimatization losses remain significant constraints. Future developments in automation, bioreactor systems, and artificial intelligence-assisted culture management may further enhance the efficiency and commercial feasibility of tissue culture technologies. Overall, tissue culture represents a key enabling technology for accelerating mulberry improvement and ensuring sustainable and climate-resilient sericulture.

Keywords: Mulberry (*Morus spp.*), sericulture, plant tissue culture, micropropagation, genetic improvement, Crispr/Cas9, germplasm conservation

Introduction

Sericulture provides rural employment and supports national economics in Asia, especially India and China (Vijayan *et al.*, 2018) ^[67]. Mulberry (*Morus spp.*) leaves are the sole food for *Bombyx mori*; leaf quality directly governs larval growth, cocoon yield and silk quality. Leaf nutrients (proteins, amino acids, sugars, moisture) critically influence growth rate, cocoon weight and filament length (Baciu *et al.*, 2023) ^[7]. The of mulberry cultivation is the higher leaf yield, better nutritive value, pest/disease and abiotic-stress tolerance, and adaptation to diverse agro-climates (Vijayan *et al.*, 2018) ^[67]. Conventional breeding has released many improved varieties but it is slow (15–20 years/variety), labor-intensive and uncertain due to high heterozygosity, dioecy, long juvenile phase and complex genetics (Sarkar *et al.*, 2017) ^[54]. Seasonal leaf production and lack of cultivars for extreme soils (saline, alkaline, flooded, acidic) remain major gaps (Dutta *et al.*, 2023) ^[17]. Through tissue culture, micropropagation, somatic embryogenesis and organogenesis enable rapid, disease-free clonal multiplication and germplasm conservation (S *et al.*, 2025) ^[48]. Molecular markers & genomics: used for germplasm characterization, QTL mapping, parent selection and marker-assisted breeding to speed varietal development (Dutta *et al.*, 2023) ^[17]. Genetic engineering / transformation: introduction of genes for drought and salt tolerance, pest/disease resistance, improved biomass and leaf quality; CRISPR and other tools promise precise trait improvement (Mangammal *et al.*, 2025) ^[38]. Integrating these tools with conventional breeding is seen as essential for climate-resilient, high-quality mulberry varieties and sustainable sericulture (Kaushik *et al.*, 2025) ^[30]. Mulberry tissue culture is now central to sericulture and mulberry

improvement. It enables high-throughput clonal propagation, secure conservation of valuable germplasm, production of clean planting material, and provides the regeneration systems required for modern genetic and metabolic enhancement of this key crop. Overall, tissue culture techniques provide a reliable alternative to traditional propagation methods by overcoming limitations like seasonal dependency and disease susceptibility, thus advancing sustainable mulberry cultivation for sericulture (Sarkar *et al.*, 2018) ^[55]. This review presents a comprehensive information about the tissue culture techniques that are used in mulberry cultivation and its applications and limitations in sericulture field.

Plant tissue culture: Concepts and principles

Plant tissue culture is the aseptic *in vitro* cultivation of plant cells, tissues, or organs on a nutrient medium under controlled conditions, enabling the growth and development of whole plants from small explants. This technique is fundamentally based on the principle of totipotency, which is the ability of a single plant cell to regenerate into a complete plant through processes like somatic embryogenesis and organogenesis (Long *et al.*, 2022) ^[36]. Gottlieb Haberlandt, who is regarded as the father of plant tissue culture, first proposed the idea of totipotency in 1902. Despite the failure of his initial experiments, later research throughout the 20th century established dependable techniques for cultivating and regenerating plants from isolated cells and tissues (Mahato *et al.*, 2023) ^[37]. Tissue culture has significant importance in perennial crops such as mulberry, which is important for sericulture and it overcomes limitations of traditional propagation methods by rapid mass multiplication, genetic improvement, disease-

free planting material production and conservation of valuable germplasm. These biotechnological approaches contribute to sustainable crop production and genetic diversity preservation, especially under challenges like climate change (Tiku *et al.*, 2021) ^[65]. Despite its advantages, tissue culture requires careful optimization due to physiological and developmental challenges encountered during *in vitro* growth (Bairu and Kane, 2011) ^[8].

Tissue culture in mulberry (*Morus spp.*)

Due to its economic significance in sericulture as the only food source for silkworms and the drawbacks of conventional propagation techniques like stem cuttings and grafting, which have poor success rates, seasonal dependence, and disease susceptibility, mulberry is an excellent choice for tissue culture. These difficulties are successfully overcome by tissue culture methods including micropropagation, somatic embryogenesis, and organogenesis, which allow for the quick, large-scale creation of healthy, disease-free planting material. The plant's ability to regenerate, particularly from nodal segments, facilitates effective aseptic establishment and good *in vitro* survival rates (Choudhary *et al.*, 2025) ^[15]. Additionally, tissue culture makes it easier to preserve and improve the genetic makeup of valuable mulberry germplasm under controlled circumstances, which is essential for maintaining productivity and adjusting to environmental stressors. (Sarkar *et al.*, 2018) ^[55]. The ability to produce triploid plants with enhanced leaf yield and quality through tissue culture further enhances mulberry's suitability for this technique (Khan and Naik, 2017) ^[31]. Overall, tissue culture provides a reliable platform for mass multiplication, genetic enhancement, and long-term preservation of mulberry varieties essential for sustainable sericulture. (Sarkar *et al.*, 2018) ^[55].

Components of tissue culture media

Plant tissue culture media consist of several essential components that support the growth and development of plant cells and tissues *in vitro*. Macronutrients such as nitrogen, potassium, phosphorus, magnesium, calcium, and sulphur provide the primary elements required for cellular functions and metabolism, while micronutrients like iron, manganese, zinc, copper, boron, molybdenum, and chlorine are needed in smaller amounts but are crucial for enzyme activities and physiological processes (Hameg *et al.*, 2020) ^[24]. Vitamins, including thiamine, nicotinic acid, pyridoxine, and myo-inositol, act as coenzymes or precursors in metabolic pathways to promote cell division and differentiation (Arteta *et al.*, 2022) ^[6]. The carbon source, like sucrose, supplies energy and maintains osmotic balance; it is most widely used sugar because it mimics the natural transport sugar in plants and supports morphogenetic processes like shoot proliferation and root induction (Yaseen *et al.*, 2013) ^[69]. Growth regulators such as auxins, cytokinins, gibberellins, and abscisic acid regulate organogenesis and somatic embryogenesis by influencing cell division, elongation, and differentiation (Pasternak and Steinmacher, 2024) ^[43]. To solidify the medium for supporting explants physically, gelling agents like agar or phytagel are used, their physical properties affect water retention and diffusion of nutrients which can impact callus growth and development (Muzika *et al.*, 2024) ^[41]. Lastly, maintaining an optimal pH (usually around 5.6 to 5.8) is

critical because it influences nutrient availability and uptake by cultured tissues.

Major Tissue Culture Techniques in Mulberry

1. Micropropagation

1.1 Nodal culture

Mulberry (*Morus spp.*) micropropagation typically uses axillary buds, nodal segments, and shoot tips as explants for *in vitro* culture. Research demonstrates that in a variety of mulberry species, such as *Morus alba* and *Morus nigra*, the addition of cytokinins like 6-Benzylaminopurine (BAP) and auxins like Indole-3-butyric acid (IBA) or Naphthalene Acetic Acid (NAA) to Murashige and Skoog (MS) medium effectively induces shoot proliferation and rooting (Munasinghe *et al.*, 2025) ^[62]. Single-node culture on cytokinin-free medium can produce longer, well-rooted shoots with larger leaves, while axillary-branching methods yield more shoots but with callus formation at the explant base (Litwinczuk and Jacek, 2020) ^[34]. Amino acids such as L-asparagine and L-glutamine are added to the medium to improve axillary bud sprouting from the nodal segment and shoot multiplication (Rahman and Islam, 2025) ^[48]. When half-strength MS medium supplemented with IBA or NAA is used, rooting success rates are typically high (70–100%), followed by acclimatisation in soil combinations with high survival rates. Molecular markers have verified the genetic integrity of micropropagated plants, confirming clonal homogeneity in propagated mulberry plantlets (Jogam *et al.*, 2018) ^[28]. Additionally, tissue culture promotes genetic development by producing triploid plants with improved leaf yield and quality and by conserving precious germplasm in controlled settings (Sarkar *et al.*, 2018) ^[55].

1.2 Auxiliary bud culture in mulberry

Auxiliary bud culture is a successful micropropagation method for mulberries that regenerates entire plants using axillary buds removed from shoot cultures. When axillary buds are encapsulated in alginate beads with additional fungicides, their survival increases and contamination decreases when they are transplanted to soil, allowing for *in vivo* growth (Bapat and Rao, 1990) ^[9]. Encapsulated axillary buds can develop shoots and roots on a variety of media, according to studies on several *Morus* species. The best shoot development is seen on Murashige and Skoog (MS) medium supplemented with benzyladenine (BA), while root formation is highest on MS basal medium without BA (Pattnaik and Chand, 2000) ^[44]. Genotypes differ in how they react to growth regulators. For example, BAP encourages sprouting in *Morus indica*, gibberellic acid (GA3) works well for *M. alba* and *M. rotundifolia*, and auxins like IBA help induce roots through auxiliary bud culture (Jain *et al.*, 1990) ^[26]. Axillary bud sprouting is also influenced by seasonal variables, with better shoot multiplication on MS medium supplemented with BAP and higher sprouting frequency throughout the summer (Chitra and Padmaja, 2002) ^[12]. Overall, axillary bud culture enables rapid clonal propagation of disease-free mulberry plants with high survival rates upon transfer to soil, supporting large-scale multiplication and genetic improvement efforts (Taha *et al.*, 2020) ^[63].

1.3 Shoot tip culture in mulberry

In mulberries, shoot tip culture is a successful micropropagation technique that allows for quick multiplication and the creation of disease-free plants.

Murashige and Skoog (MS) medium supplemented with cytokinins like 6-Benzylaminopurine (BAP) and Kinetin (Kn), frequently in conjunction with auxins like Naphthalene Acetic Acid (NAA) to promote multiple shoot formation and elongation, has been shown to produce high shoot induction frequencies (about 70–80%) (Anis *et al.*, 2003) ^[5]. Amino acids like L-asparagine and L-glutamine are added to improve axillary bud sprouting and shoot proliferation (Rahman and Islam, 2025) ^[48]. When shoots are moved to half-strength MS media supplemented with Indole-3-butyric acid (IBA) or NAA, rooting success is usually good; in certain genotypes, rooting percentages can exceed 100% (Rana *et al.*, 2022). Protocols combining chemotherapy, thermotherapy, and cryotherapy to create virus-free black mulberry plants (Elansary *et al.*, 2024) ^[19] show that shoot tip culture is especially helpful for eliminating viruses. Large-scale propagation for horticultural and sericultural uses is supported by acclimated plantlets made from branch tips, which exhibit high survival rates when transferred to soil (Taha *et al.*, 2020) ^[63].

2. Callus culture

Explants such as leaves, nodes, or shoot segments cultivated on Murashige and Skoog (MS) media supplemented with particular plant growth regulators (PGRs) are commonly used for callus induction in sericulture-related plants. Auxins like 2,4-D (2,4-dichlorophenoxyacetic acid) and NAA (1-naphthaleneacetic acid) are frequently employed in conjunction with cytokinins like benzylaminopurine (BAP) or kinetin (Kin) to effectively stimulate callus development (Ahmadpoor *et al.*, 2022) ^[2]. For example, callus has been successfully induced in a variety of species using MS medium containing 3 mg/L 2,4-D and 1-3 mg/L Kin or BAP, resulting in friable and embryogenic calli appropriate for regeneration (Kaewthip *et al.*, 2021) ^[29]. In order to minimise contamination, sterilisation procedures are essential; treatments with substances such as benomyl, NaOCl, or HgCl₂ enhance callus induction rates and explant viability (Ahmadpoor *et al.*, 2022) ^[2]. To maximise development and secondary metabolite production, callus cultures can be optimised by fine-tuning hormone concentrations and carbon sources such as maltose or sucrose (Binte Mostafiz and Wagiran, 2018) ^[10]. These methods support large-scale propagation and genetic improvement efforts in sericulture plants by providing a reliable source of undifferentiated cells for regeneration and biochemical studies (Qahtan *et al.*, 2022) ^[46]. Plant growth regulators (PGRs), which affect callus induction, growth, and secondary metabolite production, are essential in callus culture for plants connected to sericulture. *Melia azedarach* callus demonstrated the greatest induction on MS medium with 3 mg/L NAA or 2,4-D plus 1-3 mg/L Kin/BAP. Auxins like 2,4-D and NAA mixed with cytokinins like kinetin (Kin) or benzylaminopurine (BAP) are frequently used to successfully induce callus production. These PGR-supplemented callus cultures also increase the synthesis of bioactive substances like rutin and quercetin, which can promote plant resilience and health (Ahmadpoor *et al.*, 2022) ^[2]. Light conditions and carbon sources interact with PGRs to affect callus growth dynamics and quality, as seen in other plant species where combinations of BAP and NAA under specific light spectra accelerated callus emergence and shoot formation (Wijaya *et al.*, 2023) ^[68].

3. Somatic embryogenesis in sericulture

In sericulture, somatic embryogenesis (SE) is a crucial method for clonal proliferation and regeneration of plants like mulberries, which are necessary for raising silkworms. Through micropropagation and somatic embryo development, SE provides a dependable substitute for conventional propagation techniques, which have drawbacks including poor success rates and susceptibility to illness (S *et al.*, 2025). Under the influence of plant growth regulators (PGRs) like auxins, cytokinins, abscisic acid (ABA), and ethylene, which initiate signalling pathways and gene expression changes required for embryo formation, SE entails the reprogramming of somatic cells into embryogenic cells (Elhiti and Stasolla, 2022) ^[20]. By altering the developmental and stress response networks, important transcription factors such as LEAFY COTYLEDON (LEC), BABYBOOM (BBM), and SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) control the induction and development of somatic embryos (Salaun *et al.*, 2021) ^[53]. Large-scale propagation benefits from secondary somatic embryogenesis, which enables continued embryo generation from primary embryos, improving multiplication rates and sustaining embryogenic potential over time (Bogdanovic and Cukovic 2025) ^[11]. Challenges such as genotype dependence, variability in embryo quality, and maturation efficiency require optimization to ensure stable and reproducible SE protocols in sericulture crops (Gulzar *et al.*, 2020) ^[27]. Proteomic and molecular research advances are starting to untangle the intricate regulatory networks governing SE, providing opportunities for enhancing mass propagation and genetic improvement techniques in plants pertinent to sericulture (Salaun *et al.*, 2021) ^[53]. By boosting embryo yield and coordinating development, automation and liquid culture technologies utilised in other plants provide promise for expanding SE operations in sericulture crops. In general, SE can greatly increase mass propagation efforts in sericulture when paired with innovations like SSE and bioreactor culture, enabling genetic conservation and sustainable mulberry output (Egertsdotter *et al.*, 2019) ^[18].

4. Organogenesis Direct regeneration Indirect regeneration in sericulture

In plant tissue culture, organogenesis is the process of creating new organs either directly from explants (direct organogenesis) or indirectly through a callus phase (indirect organogenesis). In sugarcane, direct organogenesis from axillary buds demonstrated nearly 98% shoot formation with no discernible genetic variation (Thorat *et al.*, 2018) ^[64]. Direct regeneration usually produces shoots or roots directly from explant tissues without an intervening callus, resulting in faster propagation and higher genetic fidelity. As seen in sugarcane and other species, indirect regeneration entails callus induction followed by shoot or root production, which can result in increased genetic variability due to somaclonal variation (Alsafran *et al.*, 2022) ^[4]. Using Murashige and Skoog (MS) medium supplemented with combinations of cytokinins (e.g., 6-BA, BAP) and auxins (e.g., NAA, IBA), both direct and indirect organogenesis protocols have been developed in mulberry plants and medicinal species; direct methods typically result in higher regeneration frequencies and shorter culture times (Liu *et al.*, 2023) ^[35]. Through callus proliferation, indirect organogenesis enables mass

multiplication; nevertheless, it necessitates careful optimisation of plant growth regulators to balance shoot regeneration and callus induction (Sarropoulou *et al.*, 2023) [58]. While indirect organogenesis provides flexibility for

large-scale propagation despite some risk of variation, direct organogenesis is generally chosen for true-to-type plant production in sericulture and similar crops due to its efficiency and genetic stability (Thorat *et al.*, 2018) [64].

Table 1: Tissue Culture Techniques Used in Mulberry

Technique	Explant Source	Key Growth Regulators	Major Applications	References
Micropropagation	Nodal segments, shoot tips, axillary buds	Cytokinins (BAP, Kn), Auxins (IBA, NAA)	Rapid clonal multiplication, virus-free plants	Choudhary <i>et al.</i> , 2025; Anis <i>et al.</i> , 2003; Taha <i>et al.</i> , 2020 ^[5, 15, 63]
Callus culture	Leaves, nodes, stem segments	Auxins (2,4-D, NAA), Cytokinins (BAP, Kin)	Secondary metabolite production, indirect organogenesis	Ahmadpoor <i>et al.</i> , 2022; Kaewthip <i>et al.</i> , 2021 ^[2, 29]
Somatic embryogenesis	Leaf, cotyledon, petiole	Auxins, cytokinins, ABA	Large-scale clonal propagation, germplasm conservation	Salaun <i>et al.</i> , 2021; Bogdanovic & Cukovic, 2025 ^[11, 53]
Organogenesis (Direct & Indirect)	Nodal segments, shoot tips, callus	Cytokinins, Auxins	Shoot/root regeneration, genetic fidelity	Liu <i>et al.</i> , 2023; Thorat <i>et al.</i> , 2018 ^[35, 64]

5. Role of plant growth regulators in regeneration

In sericulture crops like mulberries, plant growth regulators (PGRs), particularly auxins and cytokinins, are essential for regeneration processes. Indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) are examples of auxins that mainly support cell elongation, root initiation, and callus formation—all of which are critical for somatic embryogenesis and indirect regeneration (Sosnowski *et al.*, 2023). Direct regeneration of shoots from explants is made possible by cytokinins such as kinetin and benzylaminopurine (BAP), which promote cell division, shoot proliferation, and organogenesis (Schaller *et al.*, 2015)

[59]. By controlling growth and stress reactions, exogenous application of these hormones improves mulberry leaf biomass and quality in sericulture, hence indirectly promoting silkworm nutrition. Auxins and cytokinins applied exogenously increased leaf biomass, protein content, and stress tolerance—all of which are essential for silkworm nutrition and cocoon quality (Suresh *et al.*, 2025) [62]. In order to optimise regeneration protocols in sericulture crops, reviews highlight that the ratio of auxin to cytokinin is crucial in guiding organogenesis pathways—greater auxin promotes root or callus development, while higher cytokinin favours shoot formation (Sosnowski *et al.*, 2023).

Table 2: Plant Growth Regulators (PGRs) Used in Mulberry Tissue Culture

PGR	Role in Tissue Culture	Typical Concentration	References
BAP (6-Benzylaminopurine)	Shoot induction, axillary bud proliferation	0.5–3 mg/L	Munasinghe <i>et al.</i> , 2019; Pattnaik & Chand, 2000 [44]
Kn (Kinetin)	Shoot elongation, organogenesis	0.5–2 mg/L	Schaller <i>et al.</i> , 2015; Liu <i>et al.</i> , 2023 [35, 59]
NAA (Naphthalene Acetic Acid)	Root induction, callus formation	0.1–2 mg/L	Rahman, 2025; Ahmadpoor <i>et al.</i> , 2022 [2, 48]
IBA (Indole-3-butyric acid)	Rooting, micropropagation	0.5–2 mg/L	Rana <i>et al.</i> , 2022; Khan & Naik, 2017 [31]
IBA (Indole-3-butyric acid)	Callus induction, somatic embryogenesis	1–3 mg/L	Binte Mostafiz, 2018; Kaewthip <i>et al.</i> , 2021 [10, 29]
ABA (Abscisic acid)	Somatic embryo maturation	0.5–1 mg/L	Elhiti & Stasolla, 2022; Salaun <i>et al.</i> , 2021 [20, 53]

6. Callus culture Media composition in sericulture

Plant growth regulators (PGRs), carbon sources, basal salts, and the physical characteristics of the culture medium all have a significant impact on callus development in sericulture plants. The nutrient composition of common basal media, such as Murashige and Skoog (MS), Gamborg's B5, Woody Plant Medium (WPM), and Linsmaier and Skoog (LS), varies and influences callus induction rates, biomass, and secondary metabolite production. For instance, it has been demonstrated that MS and WPM media support high callus growth and metabolite accumulation in a variety of species (Hazrati *et al.*, 2022) (Hazrati *et al.*, 2022) [25]. For callus induction, auxins like 2,4-dichlorophenoxyacetic acid (2,4-D) or naphthalene acetic acid (NAA) in combination with cytokinins like benzylaminopurine (BA) or kinetin are essential; the ideal concentrations vary depending on the species but typically fall between 1-3 mg/L for auxins and lower for cytokinins (Masara and Neamah, 2024) [39]. Energy and osmotic balance are provided by carbon sources, primarily sucrose at around 3%. However, different quantities of sugar or nitrogen can change callus shape, biomass, and secondary

metabolite pathways (Jan *et al.*, 2020) [27]. The physical qualities of the media are influenced by gelling agents like agar, Phytigel, or Gelrite; mechanical strength is correlated with growth characteristics and callus phenotype (Muzika *et al.*, 2024) [41]. During culture, additional additions such as casein hydrolysate or antioxidants may improve callogenesis and lessen oxidative stress or browning (Shirazi *et al.*, 2020) [60]. For callus culture to be successful, the medium composition must be carefully adjusted to balance the physical environment, hormonal signals, nutrient delivery, and stress reduction.

Applications of Tissue Culture in Mulberry Improvement

1. Synthetic seeds in mulberry

In mulberry synthetic seeds, somatic embryos are encapsulated in a protective matrix, mostly through sodium alginate technique, which, when mixed with calcium chloride, generates a gel-like coating. This encapsulation method makes it easier to handle, store, and transfer germplasm while maintaining the embryos' viability and genetic stability (Faisal and Alatar, 2019) [21]. Because of its

biocompatibility, affordability, and capacity to produce consistent beads that shield the embryos throughout storage and transportation, sodium alginate is preferred (Saeed *et al.*, 2019). Significant storage benefits are provided by synthetic seeds, such as short- to medium-term conservation at low temperatures (around 4°C), which sustains high germination rates and plantlet viability throughout time (Raju *et al.*, 2016) ^[49]. By enabling the safe exchange of rare or elite mulberry genotypes without the requirement for full plants or conventional seeds, this technique also facilitates effective germplasm movement, lowering the risks of contamination, labour, and space (Faisal and Alatar, 2019) ^[21]. Overall, synthetic seed technology provides a practical tool for clonal propagation, germplasm conservation, and large-scale dissemination in mulberry sericulture.

2. Cryopreservation of mulberry germplasm

Mulberry germplasm is cryopreserved mainly by storing it in liquid nitrogen at extremely low temperatures (-196°C), which stops metabolic processes and permits long-term conservation with little genetic changes. Mulberry germplasm is cryopreserved mainly by storing it in liquid nitrogen at extremely low temperatures (-196°C), which stops metabolic processes and permits long-term conservation with little genetic alteration. The vitrification method, which treats tissues with highly concentrated cryoprotectants (like PVS2) to prevent ice crystal formation by turning cellular water into a glass-like state, and encapsulation dehydration, which involves embedding shoot apices in alginate beads and then carefully dehydrating them before freezing, are two important cryopreservation techniques used in mulberries (Panis, 2018) ^[42]. Research indicates that while vitrification treatment length has a significant impact on survival rates, encapsulation dehydration with sucrose pretreatment and silica gel desiccation can improve explant regeneration following thawing. By dormant buds and shoot tips, these techniques sustain regeneration rates of up to about 50%, allowing for the long-term protection of rich mulberry genetic resources.

Cryopreservation is an essential tool for mulberry germplasm conservation and breeding initiatives because of its long-term advantages, which include lower space needs, a lower chance of contamination, and stable genetic integrity when compared to traditional field repositories or *in vitro* culture. (Choudhary *et al.*, 2023) ^[14].

3. Mass Clonal Propagation (Micropropagation)

Nodal segments, shoot tips and single axillary buds are used to produce thousands of true-to-type, uniform plantlets year-round (Litwinczuk and Jacek, 2020) ^[34]. Optimized MS-based media with cytokinins (BA/BAP, kinetin, mT, TDZ) and auxins (IAA, NAA, IBA) give high shoot multiplication and rooting (often >80–100% survival) in *Morus alba*, *M. indica*, *M. nigra* and others (Taha *et al.*, 2020) ^[63]. Field trials show micropropagated plants can equal or surpass cutting-derived plants in branching and leaf yield (Chitra *et al.*, 2016) ^[13].

4. Health Status: Virus-Free and Disease-Free Plants

Meristem/shoot-tip culture combined with chemotherapy, thermotherapy or cryotherapy can eliminate viruses such as black mulberry Idaeovirus, producing clean planting stock and preventing disease spread (Elansary *et al.*, 2024) ^[19]. Aseptic micropropagation inherently reduces fungal and bacterial contamination, improving nursery hygiene (Taha *et al.*, 2020) ^[63].

5. Platform for Genetic Improvement and Secondary Metabolites

Callus, organogenesis and somatic embryogenesis systems enable indirect regeneration, somaclonal variation, *in vitro* selection and are prerequisites for genetic transformation and genome editing (Long *et al.*, 2022) ^[36]. Suspension and *in vitro* cultures are used to study and enhance production of pharmacologically important secondary metabolites (phenolics, flavonoids, anthocyanins) from mulberry tissues (Baciu *et al.*, 2023) ^[7].

Table 3: Overview of Main Application Areas

Application area	Primary purpose in mulberry	Citations
Micropropagation	Rapid, uniform, year-round plant supply	Chitra <i>et al.</i> , 2016 ^[13]
Conservation	Preserve elite/rare germplasm, difficult genotypes	Vijayan <i>et al.</i> , 2011 ^[66]
Sanitation	Produce virus-free, disease-free stock	Algopishi <i>et al.</i> , 2025 ^[3]
Genetic tools & metabolites	Basis for transformation, selection, bioactive compounds	Long <i>et al.</i> , 2022 ^[36]

Somaclonal variation in mulberry

Like other plants, mulberry have both benefits and drawbacks to somaclonal variation. The primary benefit is the creation of genetic variety, which may result in the emergence of novel characteristics like stress tolerance or disease resistance that are beneficial for breeding initiatives and agricultural improvement. However, the loss of clonal homogeneity is a major drawback that makes it difficult to maintain true-to-type plants in commercial micropropagation. Molecular marker techniques, including as RAPD (Random Amplified Polymorphic DNA), ISSR (Inter-Simple Sequence Repeat), and SSR (Simple Sequence Repeat) markers, are frequently used to detect somaclonal variation. These approaches aid in evaluating genetic fidelity and identifying variants at an early stage. These markers have been widely utilised to ensure quality control in propagation systems by tracking somaclonal variation and

genetic stability in plants generated from tissue cultures. In mulberry micropropagation, somaclonal variation can be a useful tool for adding diversity, but it's important to weigh its advantages against the requirement for uniformity (Ferreira *et al.*, 2023) ^[22].

Genetic transformation in mulberry

Mulberry genetic transformation has advanced significantly, primarily through *Agrobacterium*-mediated transformation which is still the best way to introduce desired genes into mulberry tissues. This method has been refined for a range of genotypes and explants, making it possible to add genes that enhances the characteristics including pest resistance, drought and salinity tolerance, and enhanced photosynthesis (Sarkar *et al.*, 2024) ^[57]. The application of CRISPR/Cas9 genome editing is emerging as a powerful tool for precise and efficient modification of stress-responsive genes in

mulberry, offering potential to develop varieties with enhanced abiotic stress tolerance including drought, salinity, and temperature extremes (Kumar *et al.*, 2023) [33]. Stress tolerance genes like AtDREB2A and AtSHN1, which provide drought and salt resistance while lowering post-harvest water loss, have been the focus of genetic engineering efforts. This preserves leaf quality, which is essential for silkworm nutrition. Since mulberry leaf nutritional content directly influences silkworm growth and silk production, improving leaf quality for sericulture is a major objective. Molecular breeding in conjunction with genetic transformation targets characteristics like protein content, soluble sugars, and secondary metabolites to improve feed quality. (Sarkar *et al.*, 2025) [56]. Overall, modern biotechnology approaches including Agrobacterium-mediated transformation and CRISPR/Cas9 editing are accelerating mulberry improvement by enabling targeted trait enhancement for stress resilience and sericulture productivity.

Importance of mulberry improvement in sericulture through tissue culture

Mulberry improvement in sericulture depends on tissue culture techniques like micropropagation and somatic embryogenesis, which overcome the drawbacks of conventional propagation methods and allow for the mass multiplication of healthy, high-quality planting material and genetic conservation (Sarkar *et al.*, 2018) [55]. Silkworm growth, cocoon weight, and silk production are all directly impacted by leaf quality. By boosting macronutrients and bioactive substances (Mangammal *et al.*, 2025) [38], enhanced leaf nutrition by genetic transformation and molecular breeding improves these qualities. Climate-resilient genotypes and cultivation techniques like mulching and effective irrigation assist lessen the effects of climate stressors including drought, temperature extremes, and salinity, which lower mulberry leaf yield and quality and endanger silk productivity (Qayoom *et al.*, 2023) [47]. Disease susceptibility to fungi, bacteria, viruses, nematodes, and pests significantly impacts leaf yield; integrated management along with breeding for resistance is essential to sustain production (Dandin and Kumari, 2021) [16]. The development of high-yielding mulberry varieties through controlled pollination and biotechnological interventions has increased leaf yield substantially, supporting higher silk production and farmer profitability (Dutta *et al.*, 2023) [17]. Overall, combining tissue culture, genetic improvement, stress management, and disease resistance strategies is vital for sustainable sericulture with enhanced leaf quality and consistent silk production under changing environmental conditions (S *et al.*, 2025).

Limitations of tissue culture in sericulture

Tissue culture in sericulture faces several limitations despite its advantages from mass propagation and genetic improvement. One of the major challenges is the high cost of establishing and maintaining tissue culture laboratories, including expenses for media components, sterile facilities and skilled personnel. Contamination problems are very common due to the aseptic conditions required, which can lead to significant losses of cultures and increased production costs. Additionally, somaclonal variation—genetic or epigenetic alterations that take place during *in vitro* culture—could have an impact on the uniform and

quality of regenerated plants, which are essential for sericulture (Abdalla *et al.*, 2022) [1]. The process's accessibility is limited in some areas since it requires specialised workers to handle intricate protocols and fix problems. Furthermore, plantlets that are moved from controlled *in vitro* environments to natural circumstances experience acclimatisation losses, which frequently lead to significant death rates unless carefully handled using optimal hardening procedures (Purohit *et al.*, 2011) [45]. These factors collectively constrain the large-scale adoption of tissue culture in sericulture despite its potential benefits (Abdalla *et al.*, 2022) [1].

Future prospects of tissue culture in sericulture

Tissue culture in sericulture has great future potential due to developments in automation and bioreactor technologies that can greatly boost mulberry propagation's efficiency and scalability. While bioreactors allow for the large-scale generation of consistent, high-quality plantlets under regulated circumstances, supporting mass multiplication for sericulture, automation lowers the dangers of contamination and manual labour. Artificial intelligence (AI) is being incorporated into tissue culture procedures to monitor plantlet health, optimise culture conditions, and forecast results, all of which increase success rates and lower expenses (Koti and Bill, 2025) [32]. By providing precise genetic enhancements for traits like stress tolerance and leaf quality without requiring lengthy breeding cycles, genome editing technologies like CRISPR/Cas9 are anticipated to supplement tissue culture (Mangammal *et al.*, 2025) [48]. In order to maintain sericulture in the face of shifting environmental circumstances, including drought, salt, and temperature extremes, it is essential to develop climate-resilient mulberry types using a combination of tissue culture and genetic engineering techniques (Qayoom *et al.*, 2023) [47]. Overall, these innovations hold potential to transform mulberry cultivation by enhancing productivity, resilience, and sustainability in the sericulture industry.

Conclusion

The mass propagation of healthy, superior mulberry plants which are necessary for silkworm nutrition and silk production is made possible via tissue culture, which is crucial to sericulture. By offering disease-free planting material and preserving priceless germplasm, it overcomes the drawbacks of conventional propagation techniques and promotes sustainable sericulture practices. Mulberry cultivars with enhanced stress tolerance, disease resistance, and yield stability under shifting environmental conditions can be developed by combining tissue culture with molecular breeding techniques, such as genetic transformation and genome editing. In order to produce climate-resilient mulberry cultivars that can maintain sericulture in the face of biotic and abiotic challenges made worse by climate change, this combination strategy is essential. The synergy between tissue culture and advanced molecular tools promises to accelerate genetic improvement and ensure long-term productivity and economic viability of the sericulture industry. Overall, tissue culture's importance lies not only in propagation but also as a platform for innovation that supports sustainable and resilient sericulture systems through integration with modern breeding technologies.

References

1. Abdalla N, El Ramady H, Seliem MK, El Mahrouk ME, Taha N, Bayoumi Y, *et al.* An academic and technical overview on plant micropropagation challenges. *Horticulturae*,2022;8(8):677.
2. Ahmadpoor F, Zare N, Asghari R, Sheikhzadeh P. Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production in *in vitro* cultures *Melia azedarach* L. *AMB Express*,2022;12(1):3.
3. Algopishi U, Abdelghffar A, Aboud N, Safhi F, Fayad E, Alharbi K, *et al.* The genetic applications of plant cell and tissue culture techniques: Essential tools for genetic manipulation and crop improvement. *Notulae Botanicae Horti Agrobotanici Cluj Napoca*, 2025, 53(3). <https://doi.org/10.15835/nbha53314718>.
4. Alsafran M, Wickramanayake K, Usman K, Ahmed T. Efficient shoot regeneration of medicinal plant *Haplophyllum tuberculatum* by direct and indirect organogenesis and genetic fidelity assessment using Inter Simple Sequence Repeats markers. *Frontiers in Plant Science*, 2022, 13, 995825.
5. Anis M, Faisal M, Singh SK. Micropropagation of mulberry (*Morus alba* L.) through *in vitro* culture of shoot tip and nodal explants. *Plant Tissue Cult*,2003;13(1):47-51.
6. Arteta TA, Hameg R, Landin M, Gallego PP, Barreal ME. Artificial neural networks elucidated the essential role of mineral nutrients versus vitamins and plant growth regulators in achieving healthy micropropagated plants. *Plants*,2022;11(10):1284. DOI: <https://doi.org/10.3390/plants11101284>
7. Baci E, Baci G, Moise A, Dezmiorean D. A status review on the importance of mulberry (*Morus* spp.) and prospects towards its cultivation in a controlled environment. *Horticulturae*,2023;9(4):440. <https://doi.org/10.3390/horticulturae9040444>.
8. Bairu MW, Kane ME. Physiological and developmental problems encountered by *in vitro* cultured plants. *Plant Growth Regulation*,2011;63(2):101-103.
9. Bapat VA, Rao PS. *In vivo* growth of encapsulated axillary buds of mulberry (*Morus indica* L.). *Plant Cell, Tissue and Organ Culture*,1990;20(1):69-70. <https://doi.org/10.1007/bf00034760>
10. Binte Mostafiz S, Wagiran A. Efficient callus induction and regeneration in selected indica rice. *Agronomy*,2018;8(5):77.
11. Bogdanovic MD, Cukovic KB. Secondary somatic embryogenesis in plants: From cellular mechanisms to biotechnological potential. *Plants*,2025;14(22):3413.
12. Chitra D, Padmaja G. Seasonal influence on axillary bud sprouting and micropropagation of elite cultivars of mulberry. *Scientia Horticulturae*,2002;92:55-68. DOI: [https://doi.org/10.1016/s0304-4238\(01\)00279-5](https://doi.org/10.1016/s0304-4238(01)00279-5).
13. Chitra D, Chinthapalli B, Padmaja G. A comparative study on field performance of micropropagated plants and stem cutting derived plants of S 36 cultivar of mulberry (*Morus indica* L.). *Research in Biotechnology*, 2016, 7. DOI: <https://doi.org/10.19071/rib.2016.v7.2930>.
14. Choudhary R, Malik SK, Chaudhury R, Rao AA. Optimized recovery of cryostored dormant buds of mulberry germplasm. *Plants*,2023;12(2):225. DOI: <https://doi.org/10.3390/plants12020225>
15. Choudhary S, Yadav SK, Bishnoi K, Poonia MK, Rathore PK. Standardization of sterilization techniques and suitable explants for aseptic establishment in mulberry (*Morus* spp.) tissue culture. *Journal of Advances in Biology & Biotechnology*,2025;28(7):723-732. DOI: <https://doi.org/10.9734/jabb/2025/v28i72590>.
16. Dandin SB, Kumari V. Mulberry (*Morus* spp.) cultivation for sustainable sericulture. In *Mulberry*, 2021, 188-207.
17. Dutta H, Sawarkar A, Dutta S, Pradhan A, Yumnam S, Paul D, *et al.* Genomic approaches to ensure a more sustainable and productive future of mulberry for sericulture industry. *The Pharma Innovation*, 2023, 12. DOI: <https://doi.org/10.22271/tpi.2023.v12.i9v.22980>.
18. Egertsdotter U, Ahmad I, Clapham D. Automation and scale up of somatic embryogenesis for commercial plant production, with emphasis on conifers. *Frontiers in Plant Science*,2019;10:109.
19. Elansary D, GUrcan K, Roumi V, ŞimSek O. Efficacy of plant tissue culture techniques for eliminating black mulberry idaeovirus (BMIV) from infected black mulberry (*Morus nigra*). *Plants*, 2024, 13. DOI: <https://doi.org/10.3390/plants13212959>.
20. Elhiti M, Stasolla C. Transduction of signals during somatic embryogenesis. *Plants*,2022;11(2):178.
21. Faisal M, Alatar AA. *Synthetic seeds*. Springer, Cham, 2019. DOI: <https://doi.org/10.1007/978-3-030-24631-0>.
22. Ferreira MDS, Rocha ADJ, Nascimento FDS, Oliveira WDDS, Soares JMDS, Rebouças TA, *et al.* The role of somaclonal variation in plant genetic improvement: A systematic review. *Agronomy*,2023;13(3):730.
23. Ferreira MDS, Rocha ADJ, Nascimento FDS, Oliveira WDDS, Soares JMDS, Rebouças TA, *et al.* The role of somaclonal variation in plant genetic improvement: A systematic review. *Agronomy*,2023;13(3):730.
24. Hameg R, Arteta TA, Landin M, Gallego PP, Barreal ME. Modeling and optimizing culture medium mineral composition for *in vitro* propagation of *Actinidia arguta*. *Frontiers in Plant Science*,2020;11:554905.
25. Hazrati R, Zare N, Asghari Zakaria R, Sheikhzadeh P, Johari Ahar M. Factors affecting the growth, antioxidant potential, and secondary metabolites production in hazel callus cultures. *AMB Express*,2022;12(1):109. DOI: <https://doi.org/10.54389/RQVQ3199>
26. Jain A, Dandin S, Sengupta K. *In vitro* propagation through axillary bud multiplication in different mulberry genotypes. *Plant Cell Reports*,1990;8:737-740. DOI: <https://doi.org/10.1007/bf00272107>
27. Jan R, Khan MA, Asaf S, Lee IJ, Kim KM. Modulation of sugar and nitrogen in callus induction media alter PAL pathway, SA and biomass accumulation in rice callus. *Plant Cell, Tissue and Organ Culture (PCTOC)*,2020;143(3):517-530.
28. Jogam P, Shabnam A, Shukla P, Abbagani S, Ghosh M. *In vitro* regeneration and assessment of genetic fidelity of acclimated plantlets by using ISSR markers in PPR 1 (*Morus* sp.): An economically important plant. *Scientia Horticulturae*,2018;239:179-188. DOI: <https://doi.org/10.1016/j.scienta.2018.07.012>.

29. Kaewthip W, Dheeranupattana S, Junta P, Shank L. Sterile tissue preparation and callus induction of *Curcuma longa* Linn. CMUJ. Nat Sci,2021;20(3):2021062. DOI: <https://doi.org/10.12982/CMUJNS.2021.062>
30. Kaushik A, Priyadarshini P, Umadevi M, Thangamalar A, A A. Molecular breeding of mulberry for improved feed quality. Plant Cell Biotechnology and Molecular Biology, 2025. DOI: <https://doi.org/10.56557/pcbmb/2025/v26i9-109869>.
31. Khan M, Naik S. Implementation of tissue culture technique for the production of triploid plants of mulberry (*Morus L.*). International Journal of Scientific Engineering and Research,2017;5(7):445-447. DOI: <https://doi.org/10.70729/ijser171748>.
32. Koti P, Bill T. Plant tissue culture and genetic transformation in crop improvement. Journal of Bacteriology & Mycology: Open Access,2025;13(1):61-69.
33. Kumar M, Prusty MR, Pandey MK, Singh PK, Bohra A, Guo B, *et al.* Application of CRISPR/Cas9-mediated gene editing for abiotic stress management in crop plants. Frontiers in Plant Science,2023;14:1157678.
34. Litwinczuk W, Jacek B. Micropropagation of Mountain Mulberry (*Morus bombycis* Koidz.) 'Kenmochi' on cytokinin-free medium. Plants,2020;9:1153. DOI: <https://doi.org/10.3390/plants9111533>.
35. Liu Y, Zhan Y, Fu Q, Li S, Sun X, Wang Y, *et al.* Plant regeneration via somatic embryogenesis and indirect organogenesis in blue honeysuckle (*Lonicera caerulea* L.). Horticulturae,2023;9(9):996.
36. Long Y, Yang Y, Pan G, Shen Y. New insights into tissue culture plant-regeneration mechanisms. Frontiers in Plant Science,2022;13:926752. DOI: <https://doi.org/10.3389/fpls.2022.926752>.
37. Mahato A, Chaudhari G, Banjade G, Uprety S. Tissue culture and its application in modern agriculture. INWASCON Technology Magazine (i-TECH MAG),2023;5:09-11.
38. Mangammal P, Bhuvana S, Kiruba M, Vinothini N, Govindan K, Sasikumar K, *et al.* Advancement in genetic transformation of mulberry: Current trends and future directions. Plant Science Today, 2025. DOI: <https://doi.org/10.14719/pst.8167>.
39. Masara DJ, Neamah SI. Effect of chemical media components and silver nanoparticles on the induction of callus culture and accumulation of secondary compounds in *Trigonella foenum-graecum* L. Iraqi Journal of Agricultural Sciences,2024;55:111-121.
40. Munasinghe S, Priyadarshani S, Chithramali J. Establishment of micropropagation system for mulberry (*Morus alba* L.). Proceedings of the SLIIT International Conference on Advancements in Sciences and Humanities [SICASH], 2025.
41. Muzika NS, Kamai T, Williams LE, Kleiman M. Characterization of gelling agents in callus inducing media: Physical properties and their effect on callus growth. Physiologia Plantarum,2024;176(2):14312. DOI: <https://doi.org/10.1111/ppl.14312>
42. Panis B. Sixty years of plant cryopreservation: From freezing hardy mulberry twigs to establishing reference crop collections for future generations. In III International Symposium on Plant Cryopreservation,2018:1234:1-8.
43. Pasternak TP, Steinmacher D. Plant growth regulation in cell and tissue culture *in vitro*. Plants,2024;13(2):327. DOI: <https://doi.org/10.3390/plants13020327>
44. Pattnaik S, Chand P. Morphogenic response of the alginate-encapsulated axillary buds from *in vitro* shoot cultures of six mulberries. Plant Cell, Tissue and Organ Culture,2000;60:177-185. <https://doi.org/10.1023/a:1006424626433>.
45. Purohit SD, Teixeira da Silva JA, Habibi N. Current approaches for cheaper and better micropropagation technologies. International Journal of Plant Developmental Biology,2011;5(1):1-36.
46. Qahtan AA, Faisal M, Alatar AA, Abdel-Salam EM. Callus-mediated high-frequency plant regeneration, phytochemical profiling, antioxidant activity and genetic stability in *Ruta chalepensis* L. Plants,2022;11(12):1614.
47. Qayoom K, Manzoor S, Rafiqi A, Ayoub O. Biotic and abiotic stress management under climate change in sericulture. International Journal of Agricultural and Applied Sciences, 2023.
48. Rahman M, Islam S. Rapid multiplication by nodal segment and shoot tips through *in vitro* micropropagation of six mulberry (*Morus alba*) genotypes. Plant Tissue Culture and Biotechnology,2025;34(2):78826. DOI: <https://doi.org/10.3329/ptcb.v34i2.78826>. DOI: <https://doi.org/10.3329/ptcb.v34i2.78826>.
49. Raju C, Aslam A, Shajahan A. Germination and storability of calcium-alginate coated somatic embryos of mango ginger (*Curcuma amada* Roxb.). Horticulture, Environment, and Biotechnology,2016;57:88-96. DOI: <https://doi.org/10.1007/s13580-016-0096-7>.
50. Raju C, Aslam A, Shajahan A. Germination and storability of calcium-alginate coated somatic embryos of mango ginger (*Curcuma amada* Roxb.). Horticulture, Environment, and Biotechnology,2016;57:88-96. DOI: <https://doi.org/10.47772/ijriss.2022.6109>.
51. S G, G S, Aramani K, Kankanawadi N, K V. Tissue culture and its applications in mulberry propagation. Annual Research & Review in Biology,2025;40:2284. DOI: <https://doi.org/10.9734/arrb/2025/v40i82284>.
52. Saeed T, Shahzad A, Sharma S. Studies on single and double layered biocompatible encapsulation of somatic embryos in *Albizia lebbek* and genetic homogeneity appraisal among synseed derived lines through ISSR markers. Plant Cell, Tissue and Organ Culture (PCTOC),2020;140(2):431-445.
53. Salaun C, Lepiniec L, Dubreucq B. Genetic and molecular control of somatic embryogenesis. Plants,2021;10(7):1467.
54. Sarkar T, Mogili T, Sivaprasad V. Improvement of abiotic stress adaptive traits in mulberry (*Morus* spp.): An update on biotechnological interventions. 3 Biotech,2017;7(3):214.
55. Sarkar T, Mogili T, Gandhi Doss S, Sivaprasad V. Tissue culture in mulberry (*Morus* spp.) intending genetic improvement, micropropagation and secondary metabolite production: A review on current status and

- future prospects. In *Biotechnological Approaches for Medicinal and Aromatic Plants: Conservation, Genetic Improvement and Utilization*, 2018, 467-487.
56. Sarkar T, Nataraja KN, Sajeevan RS, Dhanyalakshmi KH, Doss SG, Raghunath MK, *et al.* Co-expression of AtDREB2A and AtSHN1 in transgenic mulberry (*Morus* spp.) confers tolerance to drought and salinity and reduces post-harvest water loss. *Plant Growth Regulation*,2025:105(3):773-786.
 57. Sarkar T, Ravindra KN, Sidhu GK, Doss SG, Raghunath MK, *et al.* Overexpression of phosphoenol pyruvate carboxylase gene of *Flaveria trinervia* in transgenic mulberry (*Morus* spp.) leads to improved photosynthesis rate and tolerance to drought and salinity stresses. *Plant Cell, Tissue and Organ Culture (PCTOC)*,2024:156(1):26.
 58. Sarropoulou V, Maloupa E, Grigoriadou K. Cretan dittany (*Origanum dictamnus* L.), a valuable local endemic plant: *In vitro* regeneration potential of different type of explants for conservation and sustainable exploitation. *Plants*,2023:12(1):182.
 59. Schaller GE, Bishopp A, Kieber JJ. The yin-yang of hormones: Cytokinin and auxin interactions in plant development. *The Plant Cell*,2015:27(1):44-63.
 60. Shirazi MR, Rahpeyma SA, Zolala J. A new approach to prevent hazelnut callus browning by modification of sub-culture. *Biologia plantarum*, 2020, 64(1).
 61. Shirazi MR, Rahpeyma SA, Zolala J. A new approach to prevent hazelnut callus browning by modification of sub-culture. *Biologia Plantarum*,2020:64(1):1-10.
 62. Suresh G, Sharma P, Dadrwal BK, Jat RK, Singh VP, Kumar M, *et al.* Plant growth regulators: Enhancing mulberry leaf characteristics for sustainable sericulture. *Journal of Scientific Research and Reports*,2025:31(10):722-732.
 63. Taha H, Ghazy U, Gabr A, El-Kazzaz A, Ahmed E, Haggag K. Optimization of *in vitro* culture conditions affecting propagation of mulberry plant. *Bulletin of the National Research Centre*, 2020, 44. DOI: <https://doi.org/10.1186/s42269-020-00314-y>.
 64. Thorat AS, Sonone NA, Choudhari VV, Devarumath RM, Babu KH. Plant regeneration from direct and indirect organogenesis and assessment of genetic fidelity in *Saccharum officinarum* using DNA-based markers. *Bioscience Biotechnology Research Communications*,2018:11(1):60-69.
 65. Tiku AR, Thomas TD, Razdan MK. Plant tissue culture in mulberry improvement. In *Mulberry*, 2021, 108-121.
 66. Vijayan K, Saratchandra B, Silva J. Germplasm conservation in mulberry (*Morus* spp.). *Scientia Horticulturae*,2011:128:371-379. <https://doi.org/10.1016/j.scienta.2010.11.012>.
 67. Vijayan K, Srivastava P, Raju P, Saratchandra B. Breeding for higher productivity in mulberry. *Czech Journal of Genetics and Plant Breeding*,2018:48:147-156. DOI: <https://doi.org/10.17221/162/2011-cjgpb>.
 68. Wijaya AN, Poernomo C, Savitri WD, Irawati F, Hardjo PH. The effectiveness of growth regulators and light color spectrum on callus growth of *Amorphophallus muelleri* Blume var. Madiun1. *Agromix*,2023:14(2):182-188.
 69. Yaseen M, Ahmad T, Sablok G, Standardi A, Hafiz IA. Role of carbon sources for *in vitro* plant growth and development. *Molecular Biology Reports*,2013:40(4):2837-2849. DOI: <https://doi.org/10.1007/s11033-012-2299-z>.