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Hormonal Regulation and Environmental Synergy in Mulberry Propagation: A Review of Auxin Concentrations, Timing, and Cutting Responses

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Abstract

This review evaluates the effect of rooting hormones, cutting practices, and environmental factors in improving mulberry (*Morus spp.*) propagation. A systematic synthesis of published literature from 1990 to 2025 was conducted, focusing on experimental studies that employed auxins such as indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), and related regulators in stem cuttings and nodal explants. Evidence shows that IBA consistently outperforms other auxins, with intermediate concentrations (1000–3000 ppm) producing optimal rooting and shoot growth. Rooting success is strongly influenced by the type of cutting, the quality of the substrate, and controlled environmental conditions such as humidity and temperature. Nutrient-rich substrates, such as vermicompost, can further enhance the effectiveness of rooting hormones and support the development of healthy roots. Micropropagation using nodal explants complements conventional cutting methods by enabling rapid clonal multiplication, genetic fidelity, and conservation of elite genotypes, with benzyl adenine (BA) promoting shoot proliferation and IBA enhancing root induction. Overall, findings highlight that mulberry propagation is most effective when hormone regulation is balanced with appropriate cutting practices and favorable environmental conditions. Integrating conventional cutting-based methods with micropropagation offers practical and sustainable strategies to strengthen mulberry cultivation and support sericulture.

Keywords: Adventitious root formation, Auxin environment interaction, Indole-3-butyric acid (IBA), Micropropagation, Naphthaleneacetic acid (NAA), *Morus alba*, Vegetative propagation

Introduction

Mulberry (*Morus spp.*) serves as the primary food source for silkworms, which metabolize its foliage and associated proteins into silk. High-yielding mulberry cultivars are essential for maintaining the economic viability of sericulture. The application of plant growth regulators in mulberry cultivation exhibits distinctive responses under favorable conditions. Growth promoters facilitate seed germination, stimulate sprouting, and increase leaf production. While mulberry can be propagated sexually via seeds, vegetative propagation through stem cuttings remains one of the most efficient and cost-effective approaches for clonal multiplication (Ahlawat et al., 2022). In the Philippines, mulberry cultivation uphold the viability of silk farming, yet several agronomic and structural constraints limit its potential (Caccam and Mendoza, 2015). While *Morus alba* L. is widely recognized for its adaptability and nutritional value for silkworm rearing, local production often suffers from poor leaf yield, variable quality, and inadequate propagation practices. Beyond agronomic issues, ecological challenges such

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as drought, variable soil conditions, and limited access to high-quality cultivars further aggravate production problems. These factors collectively reduce the competitiveness of Philippine silk in both domestic and international markets. Addressing these constraints requires integrated strategies that combine improved vegetative propagation techniques, rooting hormone applications, and sustainable agroforestry practices to strengthen mulberry cultivation (Ozturk et al., 2023).

Vegetative propagation is a form of asexual reproduction in which new plants arise from vegetative structures such as roots, stems, and leaves. This is important in plant breeding to maintain the original parent line for seed production (Pierik, 1997). This method plays a crucial role in the cultivation of perennial woody species, including mulberry (*Morus spp.*), a member of the family Moraceae. The genus *Morus* comprises several species distributed across temperate and subtropical regions worldwide, many of which hold significant economic and ecological importance. Among the recognized species, *Morus alba* (white mulberry), *Morus nigra* (black mulberry), and *Morus rubra* (red mulberry) are particularly notable. Of these, *M. alba* is the most extensively cultivated due to its broad adaptability to diverse climatic conditions and its indispensable role in sericulture, serving as the primary food source for silkworms (*Bombyx mori*) (Singh et al., 2014). Native to temperate Asia, *M. alba* has been widely disseminated and domesticated, resulting in substantial genetic diversity. More than 100 cultivars are recognized across the genus's 68 species, with 24 species reported in China and 19 in Japan, underscoring Asia's central role in mulberry biodiversity and cultivation (Datta, 2000).

Beyond its economic value, mulberry is also recognized for its agronomic and ecological significance. The plant exhibits rapid growth, high biomass production, and tolerance to pruning, indicating that it can withstand the removal of branches and leaves, drought, and diverse soil conditions. These attributes make mulberry highly suitable for agroforestry systems, soil conservation, and sustainable land-use practices. However, to maintain uniformity and desirable traits, mulberry is commonly propagated through vegetative methods using stem cuttings, grafting, budding, and layering, while the root parts are used to propagate in advance techniques like tissue culture, as seed propagation often results in high genetic variability (Shravankumar et al., 2023). Successful vegetative propagation in mulberry often relies on the application of rooting hormones to enhance root induction and overall plant growth. Both synthetic hormones, such as indole-3-butyric acid (IBA), Alpha-Naphthalene Acetic Acid (ANAA), Naphthalene Acetic Acid (NAA), and indole-3-acetic acid (IAA), as well as organic alternatives including aloe vera, cinnamon, and willow water, have been employed to stimulate adventitious root formation. Their use has been shown to improve rooting efficiency, increase root initiation rates, and promote balanced root and shoot development (Awotedu et al., 2021). Therefore, this review was done to synthesize current knowledge on the role of synthetic rooting hormones in the vegetative propagation of mulberry, focusing on rooting success, root quality, and plant establishment.

Moreover, this paper aimed to examine the role of rooting hormones, cutting practices, and environmental factors in improving mulberry propagation. Specifically, this study intended to: a) identify the different hormone types and concentrations used in mulberry propagation, b) recognize and assess the influence of cutting type, substrate, and environmental conditions on rooting success, and c) synthesize conventional cutting-based methods with micropropagation approaches to propose practical and sustainable propagation strategies.

Methodology

This review employed a systematic synthesis of published literature to evaluate the role of auxins and related growth regulators in mulberry (*Morus spp.*) propagation. The methodological framework was designed to ensure comprehensive coverage, critical appraisal, and thematic integration of findings across diverse experimental contexts. In line with this framework, the initial search yielded 123 full-text articles; 91 articles were assessed, and 44 studies were included in the final synthesis. Relevant studies were retrieved from peer-reviewed journals, conference proceedings, and technical reports published between 1990 and 2025. Searches were conducted in Scopus, Web of Science, and Google Scholar using combinations of keywords such as mulberry cuttings, IBA, IAA, NAA, rooting hormones, vegetative propagation, and micropropagation. Studies were considered eligible if they presented experimental investigations on mulberry propagation through stem cuttings or nodal explants, employed auxins (IBA, IAA, NAA, ABT, ANAA) at varying concentrations, and reported quantitative outcomes such as rooting percentage, root length, shoot growth, survival rate, or other relevant morphological parameters. This review consolidated thematic sections of methodological practices reported in mulberry propagation research, focusing on cutting preparation, hormone application, substrate selection, environmental regulation, and micropropagation protocols (Table 1). Comparative analysis was conducted to identify patterns, optimal ranges, and species-specific responses. Findings were synthesized to highlight consistent trends, variations across studies, and practical implications for propagation protocols. The aim was to highlight consistent approaches and identify methodological variations that influence rooting and growth outcomes.

Table 1. Summary of methodological approach in mulberry propagation

Parameter	Range/Condition Tested	Common Findings
Cutting type & size	Hardwood, semi-hardwood, softwood; 12–25 cm	Dormant hardwood cuttings showed higher rooting success.
Hormone type	IBA, NAA, ANAA, ABT-1	IBA generally outperformed NAA; optimal at 1000–3000 ppm.
Hormone concentration	100–6000 ppm	Intermediate levels (1000–3000 ppm) gave best rooting; very high doses reduced effectiveness.
Application method	Quick dip (10 s–2 min), soaking (up to 24 h)	Short immersion effective; soaking tested in specific cultivars.
Substrate	Vermicompost, peat moss–sand, coir peat, perlite, charcoal–rice husk–sand	Vermicompost and peat moss–sand improved rooting when combined with IBA.
Environmental conditions	Mist chambers, polyhouse, greenhouse; 80–90% RH; 18–32 °C	Controlled humidity and temperature critical for rooting success.
Micropropagation	Nodal explants on MS medium with BA, KIN, NAA, IBA	BA promoted shoot proliferation; IBA enhanced root induction; survival up to 80–100%.

Analytical Approach

Extracted data were synthesized qualitatively to identify consistent trends and divergences across studies. Concentration ranges were compared to determine thresholds for optimal rooting response, while environmental interactions were analyzed to highlight conditions that enhance or suppress hormone efficacy. Micropropagation protocols were reviewed separately to contrast in vitro hormone regimes with conventional cutting-based propagation.

Results and Discussion

Effects of time, rooting hormones, and concentrations for mulberry cuttings

Application of auxin has been shown to promote histological developments, including callus formation, tissue initiation, and vascular differentiation (Lavania et al., 2021). Research on mulberry propagation demonstrates that successful rooting and subsequent growth depend not only on cutting dimensions and planting depth but also on the interaction between exogenous growth regulators and nursery conditions. Standardized cutting sizes (12–15 cm length, 0.6–0.8 cm diameter) and uniform planting practices provide a consistent baseline, yet the comparative use of auxin-based regulators ABT, IBA, and IAA reveals differential effects on growth performance. Mulberry propagation trials show that indole-3-butyric acid (IBA) is more effective than other auxins in improving cutting performance. At 100 ppm, IBA consistently produced better results than naphthalene acetic acid (NAA) and indole-3-acetic acid (IAA) in sprouting, rooting, biomass growth, and shoot development (Ishfaq, 2023). This suggests that IBA supports not only improved root formation but also overall plant growth. The results highlight a clear concentration effect, where moderate doses of IBA give the best response, while similar levels of other auxins do not provide the same benefits. Overall, these findings confirm that successful mulberry propagation is best achieved with IBA, as it promotes both root and shoot growth in a balanced way. Research on auxin use in mulberry propagation shows that both concentration and substrate type strongly influence multiplication success. Singh (2018) reported that hardwood cuttings treated with 2000 ppm indole-3-butyric acid (IBA) in vermicompost achieved significantly higher multiplication rates compared with untreated controls and lower concentrations (1000 ppm). These findings support broader evidence that optimal auxin levels stimulate strong root and shoot initiation, while lower doses do not fully maximize growth. In addition, the positive interaction between IBA and nutrient-rich vermicompost highlights the importance of combining hormonal regulation with high-quality substrates. Hussein et al. (2021) conducted comparative studies on auxin application in mulberry propagation show that rooting efficiency depends strongly on both hormone type and concentration. Under controlled polyhouse conditions, 1000 ppm indole-3-butyric acid (IBA) consistently produced the highest rooting percentage in both white and black mulberry, outperforming lower and higher IBA concentrations as well as treatments with naphthalene acetic acid (NAA) at different levels. These results suggest that IBA provides a more reliable stimulus for root initiation than NAA, with its effectiveness peaking at intermediate concentrations rather than extremes. Overall, the findings highlight the need to calibrate auxin dosage to species-specific physiological responses, reinforcing the broader conclusion that successful propagation depends on balancing hormonal stimulation with environmental regulation. Furthermore, Pallavi (2018) reported that cuttings measuring 15–20 cm in length achieved improved rooting growth when treated with 2000 ppm indole-3-butyric acid (IBA) in combination with 5% phosphate-solubilizing bacteria (PSB). This outcome highlights a broader principle in vegetative propagation; appropriate cutting size ensures sufficient carbohydrate reserves and rooting potential, while the combined application of auxins and beneficial microbes accelerates root initiation and nutrient uptake.

Polat (2006) assessed local mulberry cultivars for rooting performance using hardwood cuttings 20–25 cm in length, collected during the dormant season from one-year-old shoots (January 15 in the first year and February 20 in the second year). Cuttings were immersed in indole-3-butyric acid (IBA) solutions of varying concentrations for 10 seconds, and rooting parameters, including average root length, rooting percentage, and number of roots per cutting, were recorded 93–118 days after planting. It is demonstrated that hardwood cuttings collected during dormancy responded most favorably to indole-3-butyric acid (IBA), with 5000 ppm producing the highest rooting percentage, particularly in the cultivar Beyrudi (31.7%). These findings reinforce broader evidence that auxin treatment enhances adventitious root initiation, while seasonal timing ensures physiological readiness of the cuttings (Polat, 2015). Husen et al. (2017) demonstrated that soft stem cuttings of white mulberry achieved the highest rooting response at 3000 ppm IBA, when cultured in mist propagation chambers maintained at 32/26 °C (day/night) and 85 ± 2% relative humidity.

Rooting success in mulberry has been shown to depend not only on auxin concentration but also on the propagation environment. The research findings by Harika et al. (2024) show that 3000 ppm indole-3-butyric acid (IBA) consistently produced superior rooting responses in apical shoots under mini clonal technology, particularly when coir peat and sand were used as the propagation medium. This concentration outperformed both lower and higher IBA doses (1000 ppm, 4000 ppm, and 5000 ppm) and treatments with naphthaleneacetic acid (NAA), indicating that 3000 ppm represents an optimal threshold for auxin-induced root development. The results emphasize not only the importance of selecting the appropriate hormone type but also the critical role of concentration balance, as excessive levels reduce effectiveness while moderate levels maximize rooting efficiency. Also, Vijay et al. (2023) reported that IBA at 3000 ppm was the most effective treatment for semi-hardwood cuttings of Korean mulberry, yielding higher survival, rooting, and shoot growth compared with both lower concentrations (1000–2000 ppm), higher concentrations (4000–5000 ppm), and NAA applications. This pattern suggests the existence of an optimal auxin level, where plant responses are maximized but decline when concentrations fall below or rise above this point. The findings highlight the need for careful hormone adjustment in propagation practices, rather than assuming that higher doses always lead to better results. Softwood cuttings of black and white mulberry showed complete rooting when propagated in perlite under mist conditions with 85–90% relative humidity. Root initiation was regularly observed across humidity regimes, provided rooting zone temperatures were held at 18–20 °C and ambient temperatures at 29–31 °C.

IBA applications between 1000 and 4000 ppm further supported this high rooting success, highlighting the combined importance of substrate, humidity, temperature, and hormone concentration in achieving reliable propagation outcomes (Kalyoncu et al., 2009). However, IBA concentrations at 200 and 400 ppm have been identified as suitable for mulberry propagation, offering a reliable approach for rooting success (Sourati et al., 2022). In fruitless white mulberry, one-year-old cuttings collected at three-week intervals from late February to late March revealed that the timing of collection influenced growth responses. In particular, cuttings obtained on March 1st treated with 2000 ppm IBA produced the greatest seedling height, root length, leaf number, and leaf area, underscoring the combined importance of hormone concentration and collection period in optimizing propagation outcomes (Hawramee et al., 2019). Hardwood cuttings from three-year-old red and white Pakistan mulberry trees propagated in a peat moss–sand mixture (3:1) under greenhouse conditions showed improved rooting performance. Application of 6000 ppm IBA produced greater root number and length compared with 2000 ppm and 4000 ppm treatments, indicating that higher auxin concentration enhanced root development under these conditions (Elwakeel et al., 2024). Stem cuttings of Himalayan mulberry soaked in IBA solutions for two minutes and propagated in pots covered with clear plastic bags containing a substrate mixture of charcoal, rice husk, and fine sand showed enhanced rooting performance. The highest number of roots was obtained at 3000 ppm IBA, outperforming lower concentrations of 500, 1000, and 2000 ppm. These results highlight the importance of identifying an optimal auxin level to maximize root development under controlled propagation conditions (Sokhuma et al., 2018). Hardwood cuttings of mulberry cultivars (*Morus alba* and *Morus nigra*) propagated under mist conditions showed improved vegetative performance with auxin application. Among the treatments, 3000 ppm NAA proved most effective, producing economically viable rooting and growth outcomes compared with lower IBA concentrations (500, 1000, and 1500 ppm) and both lower (2000 ppm) and higher (4000 ppm) NAA levels (Hussein et al., 2021). These results emphasize the importance of identifying an optimal hormone concentration to maximize propagation efficiency. Apical shoot cuttings of mulberry propagated under mini-clonal technology responded strongly to auxin application. Treatment with 4000 ppm NAA, as reported by Harika et al. (2024), produced a markedly higher rooting response compared with IBA at 1000, 3000, 4000, and 5000 ppm, as well as NAA at 1000, 3000, and 5000 ppm. These findings highlight the role of NAA at an optimal concentration in enhancing root development under controlled propagation systems. White mulberry cuttings collected from matured donor tree, dried, and planted in propagation pots for 60 days under a greenhouse, are a reliable protocol for the successful growth of cuttings treated with 200 ppm NAA. However, hardwood stem cuttings treated with 6000 ppm NAA enhance rooting percentage in red and white Pakistan mulberry propagated in a wooden frame inside the greenhouse (Elwakeel et al., 2024).

The two studies imply that mulberry propagation success depends on both hormone concentration and species or cutting type. Low NAA levels (200 ppm) can be effective under controlled protocols for white mulberry, while higher concentrations (6000 ppm) are needed to enhance rooting in red and white Pakistan mulberry. Sokuhama et al. (2018) stated that Himalayan mulberry stem cuttings propagated in mixtures of charcoal, rice husk, and fine sand gave the highest results in leaf width compared to 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm IBA and 1000 ppm, 2000 ppm, 3000 ppm NAA. Application of 2000 ppm naphthaleneacetic acid (NAA) resulted in increased leaf width in black mulberry (*Morus nigra*). The propagules were obtained from hardwood cuttings derived from 8-year-old plants soaked for 24 hours, then propagated in plastic bags containing a 1:1:1 mixture of sand, soil, and silt (Altahir and Elsadig, 2025). Middle-section cuttings of black mulberry established in polyethylene bags containing a 1:1 mixture of garden soil and sand provide significantly improved growth performance when treated with ANAA supplemented with thiamine. This treatment produced higher values for shoot number, shoot length, root length, and survival rate compared to cuttings treated with Hormex, NAA, or the untreated control (Santos and Galicia, 2022).

Hormonal Regulation of Adventitious Root Formation

Rooting hormones play a vital role in regulating root formation by elevating auxin levels such as IAA, IBA, and NAA, which stimulate root initiation. Auxin is responsible for the movement of carbohydrates within the plant, encourages the flow of sugars to the leaves and upper stem, while also speeding up their transport to the rooting zone and processes fuels to develop adventitious root formation in clonally propagated plants (Husen et al., 2017). In mulberry cuttings, the growth of new roots is mainly controlled by plant hormones, with auxin being the key factor that starts root formation. Applying auxins such as IBA or NAA helps cuttings root more readily by stimulating root growth at the base. Ethylene works together with auxin, especially after the cutting is wounded, which is why freshly cut stems often root more easily than ones that have been stored (Supriya and Singh, 2024). In contrast, cytokinins and gibberellins slow rooting by promoting shoot and bud growth over root formation. Stress-related hormones also play a role, such as ABA, which slows rooting when the plant is under water stress, while jasmonates can help roots start early but block later growth if their levels stay high (Ubeda-Tomás et al., 2008). Overall, good mulberry propagation depends on managing these hormone signals through proper cutting preparation, auxin use, and careful nursery practices. This shows how knowledge of hormone interactions can be applied directly to improve rooting success in practice (Qin et al., 2026).

The effects of IBA one day following excision in *Alnus glutinosa*, where the cells within the cambial zone and adjacent phloem exhibited indications of meristematic reactivation. These cells were characterized by reduced vacuolation, the presence of distinct nuclei with prominent nucleoli, and a more intense staining response. Initial meristematic divisions were detected in these regions, occurring predominantly along the periclinal plane. Concurrently, starch accumulation increased, particularly within the cortical and pith tissues as well as in the cells comprising the vascular rays (San José et al., 2012). Application of IBA promotes adventitious root development in cuttings by activating distinct induction processes. According to Van der et al. (1993), exogenous IBA application elevates endogenous IAA through conversion, enhances its activity or synthesis, increases tissue sensitivity, and thereby promotes adventitious root formation in cuttings. Application of 4000 ppm IBA in black mulberry accelerates rooting and cell division, potentially activating carbohydrate metabolism to supply energy. In the rooting zone, protein synthesis and PEP activity are essential for cell division and differentiation during adventitious root primordium initiation and development (Aziz et al., 2024). In addition, higher IBA concentrations activate mitotic genes that are generally regulated by auxins, which might stimulate cell division (Qu et al., 2019). This hormone concentration enhanced lateral growth in the basal region of mulberry cuttings and promoted the differentiation of a greater number of primordial roots. Upon external contact with cells, IBA modulates the metabolism of enzymes, carbohydrates, RNA, DNA, and proteins in the rooting zone, which can either inhibit or stimulate root growth, particularly during cell division and differentiation (Ruppert, 1974). In *Psidium guajava*, apical cuttings treated with IBA at 3000 ppm developed more roots, likely due to an optimal hormonal effect that facilitates the accumulation and downward translocation of essential internal substances. Similar responses have been observed in numerous species, where hormone treatments stimulate cambial activity and enhance rooting. Variations in dosage requirements and root quantity may be attributed to differences in cultivar and local climatic conditions (Pallavi et al., 2018). Exogenous application of indole-3-butyric acid (IBA) modulates enzyme activities and associated cofactor levels, thereby facilitating the establishment of a favorable hormone–energy equilibrium essential for the initiation and development of root primordia.

Interaction of Rooting Hormone with Environmental Factors in Mulberry Propagation

Environmental factors had a major role in rooting development because the plant physiological state can be modulated by the environment, determining how effectively the cutting can absorb, transport, and convert IBA into active auxins (Tang et al., 2024). Indole-3-butyric acid (IBA) interacts with environmental cues to regulate root development, functioning as a signaling molecule that mediates plant responses to abiotic stressors such as temperature variation, water availability, and light intensity (Jing et al., 2023). Acting synergistically with these environmental conditions, IBA promotes root initiation, increases root density, and enhances cutting survival by stimulating metabolic activity and nutrient uptake in the developing root system (Khandaker et al., 2022). In the study of Kalyoncu et al. (2009), it was found that 100 percent rooting was obtained in softwood cutting of black and white mulberry by a humid environment (27 °C temperature, 85-90% humidity and 50% brightness) and application of IBA while 80 to 95 percent humidity can help cuttings to minimizes transpiration and prevent dehydration while they develop roots. The first findings were aligned with the study observed by Pallavi et al. (2018), which stated that variation in hormone concentrations in response to rooting may be influenced by varietal and climatic differences at the propagation site, as these factors regulate hydration, metabolic activity, and pathogen pressure during cutting propagation. Exogenous hormones such as IAA, IBA, and ABT-1 interact closely with environmental conditions to determine rooting success. Moisture, temperature, and pathogen control shape the plant's ability to absorb and respond to auxins, while stress factors like drought or excess light elevate ABA and JA, which suppress rooting. Optimal rooting occurs when hormone treatments are paired with favorable environments that sustain auxin activity and reduce inhibitory signals. Thus, rooting is best understood as a synergy where hormones provide the biochemical trigger and the environment ensures effective physiological response (Chen et al., 2023). The controlled greenhouse misting system maintains humidity and temperature, preventing desiccation and allowing IBA to stimulate root primordia formation. Seasonal timing during dormancy reduces endogenous auxin activity, exogenous IBA compensates, while genotype and mother plant age further modulate rooting outcomes, showing that hormone efficacy is contingent on environmental and physiological context (Polat, 2015).

Micropropagation of Mulberry through Nodal Explants

Micropropagation and cutting-based propagation serve as complementary approaches in addressing different limitations in plant multiplication. Cuttings-based propagation is inexpensive and widely used by farmers due to its suitability for large-scale cultivation, which allows rapid, cost-effective, and consistent cloning of superior plant varieties. While micropropagation uses tissue culture techniques to produce a large number of uniform disease-free plants in controlled conditions, together they form a twofold plan: cuttings are practical for routine small-scale propagation, while micropropagation ensures mass production, conservation of rare species, and a reliable supply when cuttings are inefficient or unavailable. This approach ensures planting material is enabled to succeed in any year-round propagation for species that are challenging to propagate through conventional methods. Micropropagation supports consistent crop performance, conservation of elite genotypes, and improved field establishment. Findings from an experiment in *Morus alba* using in vitro regeneration from nodal explants show that phytohormones regulate both shoot and root formation. The findings indicate that sustainable mulberry cultivation relies not on selecting a single method, but on integrating both approaches to ensure efficiency, genetic stability, and adaptability. Mim et al. (2024) reported that hormone regimes in mulberry micropropagation can influence the plant growth performance. Moderate concentrations of benzyl adenine (BA) stimulate shoot and leaf proliferation, while the combination of kinetin (KIN) with naphthaleneacetic acid (NAA) enhances root induction. This interplay highlights the complementary functions of cytokinins and auxins in coordinating shoot and root development, rather than relying on a single regulator. Concurrently, explant selection and sterilization protocols also critically influence in vitro success. Ellenita et al. (2020) observed that nodal segments of Thar Lohit mulberry exhibited higher survival rates (65.33%) and lower contamination (34.67%) compared to meristem tips and apical buds, reflecting their superior regenerative potential. Sequential sterilization using 70% ethanol followed by 0.2% $HgCl_2$ proved particularly effective, underscoring the necessity of optimizing both sterilant type and exposure duration to establish reliable, disease-free cultures.

Micropropagation of mulberry using nodal explants has emerged as a reliable method for rapid clonal propagation, combining both efficiency and genetic fidelity. Rahman and Islam (2024) described an efficient protocol for mulberry micropropagation utilizing nodal explants. The explants were surface-sterilized and cultured on MS medium supplemented with varying concentrations of BAP, NAA, and Kn, with subculturing performed every 21–28 days. Optimal shoot proliferation was obtained on MS medium containing 2.0 mg/L BAP and 0.2 mg/L NAA in combination with L-asparagine and L-glutamine, yielding up to 6.5 shoots per culture with an average length of 4.8 cm. Root induction was most effective on half-strength MS medium supplemented with 1.0 mg/L IBA, achieving 100% rooting with the longest roots. Acclimatized plantlets exhibited approximately 80% survival, demonstrating that nodal explants serve as a reliable and rapid source for clonal propagation of mulberry. Nodal explants of mulberry were highly effective for in vitro propagation. Cultures established on MS medium supplemented with 3% sucrose and 2.5 µM benzyl adenine achieved up to a 13-fold multiplication rate, measured as the number of nodes per shoot. Multiplication varied among seedlings and by node position, with basal nodes exhibiting more vigorous growth. Explants could be cold-stored at 4°C for up to six months without compromising their multiplication potential, and shoots rooted spontaneously after four weeks. The addition of activated charcoal further enhanced rooting and minimized callus formation. Rooted shoots transplanted into sand vermiculite mixtures exhibited 100% survival, demonstrating the robustness and efficiency of this protocol for clonal propagation of mulberry (Sharma and Thorpe, 1990).

Role of soil and substrate in mulberry cultivation

Soil quality supports sustainable mulberry cultivation, offering support, nutrients, and a habitat for microbes. Deep-rooted mulberry trees thrive in fertile, well-structured soils with good water retention, ensuring nutrient-rich foliage essential for silkworm rearing. Kumar et al. (2024) found that mixing soil with farmyard manure (FYM) creates a much better environment for root growth than using soilless substrates. In their study, the best results came from combining soil + FYM with indole-3-butyric acid (IBA) at 3000 ppm. This treatment gave the highest sprouting rate, better survival, more leaves, and stronger root growth in mulberry cuttings (Sarkar et al., n.d.). The findings show that organic manure and auxin work together in a positive way to boost plant performance.

Conclusion

The review literature shows that mulberry propagation success is strongly influenced by the type and concentration of auxins, particularly indole-3-butyric acid (IBA). Across different cutting types and propagation environments, IBA consistently produced higher rooting percentages and improved shoot growth compared with other auxins. Moderate concentrations, often around 2000 ppm and 3000 ppm, were shown to be most effective, while both lower and higher doses reduced efficiency. The findings also highlight that rooting outcomes are not determined by hormones alone, but by their interaction with environmental factors such as humidity, temperature, and substrate quality. Trials demonstrated that nutrient-rich substrates, including vermicompost and farmyard manure, enhanced the positive effects of IBA, supporting stronger root initiation and plant survival. Seasonal timing further influenced rooting success, with dormant-season cuttings responding more favorably to auxin treatments. Micropropagation studies complement these results by showing that nodal explants under controlled tissue culture conditions can provide uniform, disease-free planting material. Together, conventional cutting propagation and micropropagation offer practical and scalable solutions for mulberry cultivation. The integration of hormone regulation, environmental control, and substrate management provides a reliable framework for improving propagation efficiency. These findings contribute to the development of standardized protocols that can support both small-scale farmers and large-scale production systems. Overall, the review paper confirms that careful adjustment of hormone concentration, combined with appropriate environmental and substrate conditions, is essential for achieving consistent and sustainable mulberry propagation outcomes.

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