

Review Article

Advances in Propagation of Mulberry (*Morus* spp.): Role of Plant Growth Regulators, Rooting Substrates and Micropropagation Techniques

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Abstract

Mulberry (*Morus* spp.) is a fast growing woody perennial fruit crop that has its principal role in the sericulture sector as being its major food source of nutrition for the silkworm (*Bombyx mori*). Although a number of techniques for its propagation are available, its propagation through hardwood cuttings has go on most popular owing to its ease of operation, cheap cost of operation and result uniformity. However, successful rooting of mulberry cuttings has often being affected by a number of parameters like usage of PGRs, rooting substrate type and time of planting. The review focuses on evaluating the contribution of different PGRs more particularly indole-3-butyric acid (IBA), naphthalene acetic acid (NAA) and cytokinins in inducing root initiation and vegetative growth of mulberry cuttings. Among these PGRs, IBA was documented to be most efficient auxin of its own class because different studies indicated increased sprouting, number of root and survival rate of rooted cuttings on different genotypes of mulberry. The review has also enabled elucidation of role of rooting substrates to a larger extent; cocopeat, perlite, vermiculite, vermicompost and Farm Yard Manure mix showed superior rooting performance owing to their superior physical and chemical properties. In addition, improvement of micro-propagation techniques was also evaluated and documented to be efficient in terms of utilization of BAP and IBA for in vitro multiplication of shoot and root growth, while offering a substitute for rapid multiplication of elite genotypes. However, their limitation like genotype specific response to hormone, low rate of acclimatization and erratic field performance were reported. Based on these study, this review recommends optimizing of variety-specific hormone media combination, standardization of micro-propagation regime and integration of molecular tools for precision in propagation for improvement of propagation efficiency, with genetic fidelity of planting material and ensuring sustainable production of mulberry for a more resilient sericulture sector.

Keywords

IBA, Media, Sericulture, Cuttings, Micro-propagation, Survival Rate, Propagation, Sustainable Cultivation

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1. Introduction

Mulberry (*Morus* spp.) is an important perennial fruit tree species belongs to the Moraceae family, originating in Asia. Known for its rapid growth, this deciduous woody perennial can develop into a medium-sized tree or shrub with a robust deep-root system. Mulberry serves as an important economical and medicinal plant, commercially grown for the use of leaves as a food source of monophagous insect silkworm (*Bombyx mori*), a key role in the silk industry [28, 29]. Most species within the *Morus* genus are diploid, possessing 28 chromosomes, with the genus encompassing 68 species and over 100 cultivars [23]. These species are widely spread across Asia, with 19 species in Japan and 24 in China. Global distribution is exhibited by notable cultivars, including *Morus alba* (white mulberry), *Morus rubra* (red mulberry) and *Morus nigra* (black mulberry). Fruiting occurs in July, and the fruits can be white, purple and red. These fruits are rich in essential nutrients beneficial for human health [57]. Beyond its use as food for silk worms, mulberry wood is utilized in making agricultural tools, recreational items and furniture, while its bark is used in high-quality paper production [15]. China is the world's largest producer of silk, generating about 50,000 metric tons annually, followed by India with approximately 36,582 metric tons [42]. Mulberry plants can reproduce both sexually and asexually. Desired features of the parent plant can be preserved by asexual reproduction using stem cuttings. Propagation with semi-hardwood cuttings is the most used asexual approach due to its cost-effectiveness, rapidity and efficiency. This technique ease of use and high success rate make it frequently select over alternative methods of transmission.

Synthetic plant growth regulator (auxins), such as Indole-3-butyric acid (IBA) has gained prominence for their ability to stimulate root initiation and development in plant cuttings. It enhances rooting success by promoting cell division and elongation in root primordial, making it a key component in the propagation of mulberry cuttings. This method not only ensures higher rooting efficiency but also contributes to the overall growth and success of the propagated plants when combined with suitable auxin treatment such as IBA and NAA [28, 29]. Cytokinins, especially 6-benzylaminopurine (BAP), play an important role in plant propagation through rapid cell division, multiple shoot initiation and suppression of apical dominance that triggers lateral bud growth, for the purpose of efficient clonal multiplication. They accomplish the induction of shoot organogenesis in callus tissues, increase adventitious bud formation, delay chlorophyll degradation and improve establishment and vigor in in vitro cultures. Morphogenesis is controlled by the cytokinin-auxin ratio; high levels of cytokinin promote shoot regeneration and establish BAP as one of the major components in micropropagation for many horticultural crops [1].

Selection an appropriate growing media is essential for effective proliferation. Sand, while chemically inert and neutral in pH, lacks nutrients, higher compactness and low water absorption capacity. Cocopeat, an organic material, is appreciated for its high water absorption capacity and ion exchange properties, which help maintain pH and electrical conductivity as well as provide good porosity and proper aeration for roots. Perlite is widely recognized for its ability to enhance soil aeration and support plant growth due to its excellent water absorption properties [14, 28, 29, 46]. As well as similarly, vermiculite is valued for improving soil aeration, retaining moisture and providing essential nutrients such as nitrates, potassium, phosphorus, calcium and magnesium. For best outcomes, one year old hardwood cuttings approximately 20-25 cm in length should be planted in February or March (spring season) to achieve quicker, maximum survival rate, and more efficient rooting [28, 29].

Micro-propagation/tissue culture has revolutionized horticulture crop propagation by enabling rapid multiplication in a controlled environment. This technique involves growing plant cells/tissues in a nutrient medium under sterile conditions. Its advantages include rapid multiplication of elite varieties year-round, disease elimination and maintaining genetic stability. Micro-propagation conserves space, making it suitable for commercial production in limited areas and preserving rare varieties. Through growth condition adjustment enhances propagation efficiency. Overall micro-propagation ensures speed, disease control, genetic stability and space efficiency in fruit crop propagation, its global demands for high-quality produce [30].

- 1) The brief review of diver's experimental findings various aspects reviewed under following headings/ sub-headings.
- 2) Influence of plant growth regulators on shoot biomass response and root development in mulberry.
- 3) Influence of suitable growth media on shoot biomass response and root development in mulberry.
- 4) Influence of timing and method on shoot biomass response and root development in mulberry.
- 5) Micro-propagation on shoot biomass response and root development in mulberry.

2. Influence of Plant Growth Regulators

2.1. Shoot Emergence and Vegetative Growth

2.1.1. Number of Days Taken for Sprouting

Indole butyric acid @ 2000 ppm induced early sprouting in mulberry cuttings [29]. While conducting an experiment to study the effect of Indole butyric acid on rooting of hardwood cuttings of pomegranate, observed that cuttings treated with

IBA significantly exhibited early sprouting [8]. Studied the effect of IBA treatments on the rooting of peach hardwood cuttings with IBA @ 3000 ppm was impactful in number of days taken for sprouting [21]. The effect of IBA on propagation of fig (*Ficus carica*) by hardwood cutting treated with IBA @ 2500 ppm was impactful in number of days taken for sprouting [33].

2.1.2. Number of Sprouts Per Cutting

The cuttings of mulberry cultivar Thar Harit treated with IBA @ 2000 ppm had the maximum number of sprouts per cuttings [29]. While evaluating the effect of indole butyric acid on V-1 mulberry cultivar cuttings, noticed that maximum numbers of sprouts were recorded with IBA @ 3000 ppm [38]. Studied the effect of IBA on *Morus alba* hardwood stem cuttings treated with IBA @ 150 ppm and recorded the maximum number of sprouts per cutting [24]. The cuttings of fig cultivar Brown Turkey treated with IBA @ 1000 ppm had the maximum number of sprouts per cuttings [7]. Carried out an experiment to study the effect of IBA concentration on rooting of pomegranate cuttings and revealed that the highest numbers of sprouts per cutting were observed in cuttings treated with IBA @ 500 ppm [31]. The number of sprouts were recorded maximum in stem cuttings of star gooseberry (*Phyllanthus acidus*) with IBA @ 2000 ppm treatment [36].

2.1.3. Sprouted Cuttings Percentage (%)

The cuttings of mulberry treated with IBA @ 2000 ppm had the maximum percentage of sprouted cuttings [29]. While evaluating the influence of IBA on rooting and growth of *Morus alba* stem cuttings, found that cuttings treated with IBA @ 100 ppm had positive effect with regard to sprouting percentage [39]. Conducted an experiment to study the effect of IBA concentrations on the rooting of peach hardwood cuttings [21]. The maximum number of sprouted cuttings were noticed in IBA @ 3000 ppm. Reported the efficacy of indole butyric acid on rooting of stem cuttings of star gooseberry (*Phyllanthus acidus*) and revealed that maximum sprouting percentage was noted in cuttings treated with IBA @ 2000 ppm individually as well as in their combinations [36]. The hardwood cuttings of wild fig treated with IBA @ 2500 ppm had the maximum percentage of sprouted cuttings [33].

2.1.4. Survival Percentage of Sprouted Cutting (%)

The cuttings of mulberry treated with IBA @ 2000 ppm had the highest survival per cent [29]. While evaluating the response of IBA on stem cuttings of mulberry cultivar V-1, noted that highest survival percentage was found in stem cutting treated with IBA @ 3000 ppm [38]. Carried out an experiment to study the effect of IBA on rooting of pomegranate hardwood cuttings treated with IBA @ 3000 ppm had the highest survival percentage [8]. While studying the effect of IBA on *Morus alba* hardwood stem cuttings, revealed that the maximum survival percentage was observed in the hardwood

stem cuttings treated with IBA @ 750 ppm [24]. Studied the effect of various concentrations of IBA on the rooting of hardwood cuttings of *Morus alba* and reported that the cuttings treated with IBA @ 2000 ppm performed the best in survival percentage [15]. The cuttings of 'Himalayan' mulberry treated with IBA @ 3000 ppm had the highest survival rate [54]. While evaluating the response of IBA on hardwood cuttings of phalsa, noted that highest survival percentage was found in stem cutting treated with IBA @ 2000 ppm [51]. Carried out an experiment to study the effect of IBA on rooting of fig (*Ficus carica*) hardwood cuttings treated with IBA @ 2000 ppm and observed the highest survival percentage [6]. The cuttings of wild fig treated with IBA @ 2500 ppm had the highest survival rate [33].

2.1.5. Stem Height (cm)

The plant height was recorded maximum in the cuttings of mulberry cultivar V-1 treated with IBA @ 3000 ppm [38]. While studying the effect of different treatments of IBA on the morphological performance of hardwood stem cuttings of mulberry, found that the cuttings treated with IBA @ 2000 ppm had the highest plant height [29]. Carried out an experiment to study the effect of IBA on rooting of *Morus alba* hardwood cuttings treated with IBA @ 2000 ppm and recorded the highest stem height [15].

2.1.6. Stem Diameter (mm)

The stem diameter of mulberry was recorded highest in the hardwood stem cuttings treated with IBA @ 3000 ppm treatment [32]. While studying the effect of indole butyric acid concentrations on the rooting of mulberry cuttings, revealed that cuttings of mulberry treated with IBA @ 2000 ppm showed good results on stem diameter [29]. Reported the influence IBA on rooting and growth of pomegranate cultivar Ganesh and found that cuttings treated with IBA @ 2000 ppm had the maximum shoot diameter [49].

2.1.7. Number of Leaves Per Plant

The numbers of leaves were found maximum in hardwood stem cuttings treated with IBA @ 2500 ppm of wild fig [33]. While studying the effect of indole butyric acid concentrations on the rooting of fig cultivar Brown Turkey cuttings, revealed that cuttings of fig cultivar Brown Turkey treated with IBA @ 1000 ppm showed good results on number of leaves per cuttings [7]. Conducted an experiment to study the effect of IBA concentrations on the rooting of mulberry cultivar V-1 cuttings the maximum number of leaves were noticed in IBA @ 3000 ppm [38]. Studied the influence of IBA on rooting and vegetative growth of mulberry cuttings, the maximum number of leaves were recorded with application of IBA @ 3000 ppm [32]. The cuttings of *Morus alba* treated with IBA @ 2000 ppm had the highest number of leaves [15]. While evaluating the response of IBA on 'Himalayan' mulberry stem cuttings, noted that highest number of leaves in stem cutting treated

with IBA @ 3000 ppm [54]. The cuttings of mulberry treated with IBA @ 2000 ppm had the highest number of leaves [29].

2.1.8. Fresh and Dry Weight of Shoots (g)

The fresh and dry weight of shoots was noted highest in cutting of mulberry treated with IBA @ 2000 ppm [28]. While evaluating the effect of IBA on hardwood cuttings of fig cultivar Brown Turkey, observed that IBA @ 1000 ppm showed the maximum fresh and dry weight of shoot [7]. Conducted an experiment to study that the effect of IBA induced rooting of *Morus alba* hardwood cuttings and observed that the cuttings treated with IBA @ 2000 ppm had the maximum fresh and dry weight of shoots [15].

2.1.9. Number of Roots Generated Per Cutting

The hard wood cuttings of wild fig treated with IBA @ 2500 ppm had the highest number of roots per cutting [33]. While evaluating the effect of IBA on rooting of black mulberry hardwood cuttings, found that hardwood cutting of mulberry treated with IBA @ 2000 ppm showed the maximum number of roots per cutting [28]. While studying the effect of indole butyric acid concentrations on the rooting of black mulberry cuttings, noted that cuttings of black mulberry treated with IBA @ 6000 ppm had the highest number of roots per plant [20]. The effect of indole butyric acid on hardwood stem cuttings of *Morus alba* [24]. They found that maximum number of roots per cutting were recorded in cuttings treated with IBA @ 2000 ppm. While evaluating the effect of IBA on rooting of hardwood cuttings of black mulberry, noted that the cuttings treated with IBA @ 5000 ppm had the best result with respect to root number [11].

The cuttings of *Morus alba* treated with IBA @ 3000 ppm had the maximum number of roots [39]. While studying the effect of IBA on root and shoot development of 'Himalayan' mulberry stem cuttings, observed that highest number of roots were found in stem cuttings treated with IBA @ 3000 ppm [54]. The response of IBA on stem cuttings of mulberry the maximum number of roots per cutting were found in stem cutting treated with IBA @ 3000 ppm [17].

2.2. Root Development

2.2.1. Fresh and Dry Weight of Roots Per Cutting (g)

The hardwood cuttings of wild fig treated with IBA @ 2500 ppm showed the maximum fresh and dry weight of roots [33]. While conducting an experiment to study the effect of IBA concentration on rooting of hardwood cuttings of mulberry (*Morus alba* L.), found that cuttings treated with IBA @ 2000 ppm had the maximum fresh and dry weight of roots [15]. While studying the effect of various concentration of IBA on rooting of cuttings of fig cultivar Brown Turkey, found that the cuttings treated with IBA @ 1000 ppm showed the highest fresh and dry weight of roots [7].

The hardwood cuttings of peach treated with IBA @ 3000 ppm showed the maximum fresh and dry weight of roots [21]. While conducting an experiment to study the effect of IBA concentration on rooting of mulberry cuttings, found that cuttings treated with IBA @ 2000 ppm had the maximum fresh and dry weight of roots [28]. While studying the effect of various concentration of IBA on rooting of fig cuttings, found that the cuttings treated with IBA @ 2000 ppm showed the highest fresh and dry weight of roots [5, 6].

2.2.2. Root to Shoot Ratio

The cuttings of phalsa treated with IBA @ 300 ppm showed the maximum root to shoot ratio [41]. An experiment was conducted to study the effect of indole butyric acid on rooting of stem cuttings of star gooseberry. In this study IBA @ 2000 ppm individually as well as in their combination had the maximum root to shoot ratio [36]. While studying the effect of IBA treatments on hardwood cuttings in mulberry cultivar Thar Harit, found that cuttings treated with IBA @ 2000 ppm had the highest root to shoot ratio [28].

2.2.3. Root Diameter (mm)

The root diameter was recorded highest in hardwood cutting of black mulberry treated with IBA @ 6000 ppm [34]. While studying the effect of IBA on the rooting of black mulberry (*Morus nigra* L.) hard wood cuttings, found that maximum root diameter was recorded in IBA @ 6000 ppm [20]. The effect of various concentrations of IBA on the rooting of fig cutting and reported that the cuttings treated with IBA @ 2000 ppm had the maximum root diameter [27]. The effect of IBA on rooting of hardwood cuttings of phalsa and found that cuttings treated with IBA @ 2000 ppm had the maximum root diameter [51]. The effect of IBA on rooting of lemon cuttings and found that cuttings treated with IBA @ 2000 ppm had the maximum root diameter [53]. The effect of various concentrations of IBA on the rooting of pomegranate stem cuttings and reported that the cuttings treated with IBA @ 2000 ppm had the maximum root diameter [9]. Studied the effect of various concentrations of IBA on mulberry cuttings the highest root diameter was reported with IBA @ 2000 ppm [28].

2.2.4. Length of the Longest Root (cm)

The root length was recorded highest in hardwood cutting of wild fig treated with IBA @ 2500 ppm [33]. While conducting an experiment for the effect of IBA on the rooting of black mulberry hardwood cuttings, observed that the cuttings treated with IBA @ 6000 ppm had the maximum root length [34]. Studied the response of IBA on hard wood cuttings of black mulberry (*Morus nigra* L.) and observed that maximum length of the longest root with IBA @ 6000 ppm [20]. Conducted an experiment to study various concentrations of IBA on hardwood stem cuttings of *Morus alba* and observed that maximum length of root was obtained when cuttings treated

with IBA @ 2000 ppm [28]. The effect of various concentrations of IBA on the rooting of hardwood cuttings of black mulberry (*Morus nigra* L.) and reported that the cuttings treated with IBA @ 5000 ppm had the highest root length [11].

The root length of *Morus alba* was recorded highest when hardwood cuttings treated with IBA @ 2000 ppm [15]. While studying the effect of various IBA concentrations on root and shoot development of 'Himalayan' mulberry stem cuttings, found that root length was maximum in IBA @ 3000 ppm [54]. An experiment to study the effect of IBA on the rooting in stem cutting of mulberry and observed that maximum length of root was recorded with IBA @ 3000 ppm [17].

2.2.5. Number of Roots

The numbers of primary and secondary roots were found maximum in hardwood cuttings of *Morus alba* treated with IBA @ 2000 ppm [43]. While conducting an experiment to study the effect of IBA on the rooting in stem cutting of mulberry (*Morus nigra* L.) found that the maximum number of primary and secondary roots were recorded with IBA @ 2000 ppm [50]. The various concentrations of IBA on rooting of hardwood cuttings of mulberry (*Morus alba* L.) and found that the maximum number of primary and secondary roots was recorded with IBA @ 5000 ppm [53]. Conducted an experiment and observed the different concentrations of IBA on mulberry cuttings. The highest numbers of primary and secondary roots were reported with IBA @ 2000 ppm [28].

2.3. Physiological and Nutritional Response Nutrients Status of Leaves

The nitrogen, phosphorus, potassium and chlorophyll content were found maximum in hardwood cuttings of mulberry treated with IBA @ 2000 ppm compared to control [28].

3. Influence of Suitable Growth Media

3.1. Shoot Growth and Survival

3.1.1. Number of Sprouts Per Cutting

The number of sprouts per cutting were significantly increased in hardwood cuttings of mulberry planted in soil, sand and FYM in a ratio of 1:1:1 [52]. While conducting an experiment to study the effect of potting media on growth and development of semi-hardwood cuttings of fig (*Ficus carica*) cv. Black Ischia found that the mixture of pond soil, river sand and vermicompost (2:1:1) was the most effective treatment which showed better results in number of sprouts per cutting [25]. While conducting an experiment to study the effect of various potting media on growth and development of stem cuttings of mulberry (*Morus nigra* L.) found that sand, FYM, vermicompost and cocopeat was the most effective treatment which showed better results in number of sprouts per cutting

[54]. The number of sprouts per cutting were significantly increased in hardwood cuttings of mulberry planted in growing media combination of cocopeat, perlite and vermicompost in a ratio of 2:1:1 [29].

3.1.2. Sprouted Cuttings Percentage (%)

The mulberry cuttings planted in rooting media soil, FYM and coir pith (1:1:1) had the maximum sprouted percentage [55]. While investigating the effect of rooting media on survival of hardwood stem cuttings of lemon cultivar Pant Lemon-1 found that maximum sprouting percentage was recorded in soil + sand + cocopeat medium [52]. The effect of suitable growing media on mulberry propagation through cutting and observed that among all the rooting media, the highest sprouting success was recorded in the combinations of cocopeat, perlite and vermicompost in a ratio of 2:1:1 [29].

3.1.3. Survival Percentage of Sprouted Cutting (%)

The highest percentage of survival was recorded in stem cuttings of *S. compacta* species treated with IBA @ 2000 ppm and planted in perlite and peat of a equal (1:1) ratio [3]. Rooting response of cuttings of mulberry in rooting media observed that the cuttings planted in soil, FYM and coir pith (1:1:1) had the highest survival percentage [54]. An experiment to study the effect of different rooting media on semi-hardwood cuttings of fig (*Ficus carica*) cv. Black Ischia and results revealed that pond soil, river sand and vermicompost (2:1:1) showed the maximum survival percentage of rooted cuttings [25]. The rooting medium in a soil, red soil, vermicompost in ratio 2:1:1 on the rooting of dragon fruit showed better results in survival percentage [18]. Rooting response of cuttings of mulberry in suitable growing media, Meena et al. (2026_b) observed that the cuttings planted in the combinations of cocopeat, perlite and vermicompost in a ratio of 2:1:1 had the highest survival percentage.

3.1.4. Stem Height (cm)

The plant height of fig (*Ficus carica*) cv. Black Ischia was recorded maximum in growing media pond soil, river sand and vermicompost in a ratio of 2:1:1 [25]. While conducting an experiment on rooting medium of perlite and peat mixture in equal volume 1:1 ratio on the rooting of stem cuttings of *S. compacta* species, Arslan, (2024) found that maximum plant height in perlite and peat mixture of equal volume ratio. Reported that the substrate mixture comprising cocopeat, perlite and vermicompost in a 3:1:1 ratio, when used in large sized earthen pots, resulted in a greatest shoot length (23.60 cm) and the highest root-shoot ratio (0.67) in strawberry plants [12]. Studied the response in strawberry growth in different substrates and containers under greenhouse and found that maximum plant height was observed in cocopeat, perlite and vermicompost (3:1:1) [46]. While conducting an experiment on rooting medium of cocopeat, perlite and vermicompost in a ratio of 2:1:1 on the rooting of mulberry cuttings, found that

maximum plant height [29].

3.1.5. Stem Diameter (mm)

The rooting medium in 1:1 ratio of perlite and peat mixture on the rooting of stem cuttings of *S. compacta* species showed better results in stem diameter [3]. While conducting an experiment rooting medium of equal volume soil to sand mixture on the rooting of stem cuttings of salix, [48]. found that maximum stem diameter. The effect of different suitable growing media and found that the maximum stem diameter were observed with cutting planted in the combinations of cocopeat, perlite and vermicompost in a ratio of 2:1:1 [29].

3.1.6. Number of Leaves/Plants

The rooting medium in a soil, sand and vermicompost (1:1:1) on rooting of *M. indica* variety V1 showed better results in number of leaves/plant [54]. While studying the effect of types of rooting medium on rooting of semi-hardwood cuttings of fig (*Ficus carica*) cv. Black Ischia observed that number of leaves per cutting were significantly increased when hardwood cutting planted in pond soil, river sand and vermicompost (2:1:1) medium [25]. The effect of different rooting media and found that the maximum number of leaves was observed with cutting planted in sand, FYM and clay [43]. The response in mulberry growth in different substrates under open condition and found that maximum number of leaves was observed in cocopeat, perlite and vermicompost (2:1:1) [29].

3.1.7. Fresh and Dry Weight of Shoot (g)

The fresh and dry weight of mulberry shoot, (*Morus alba* L.) was recorded maximum with potting media soil, sand and farmyard manure in a ratio of 1:1:1 [52]. While conducting an experiment to study the effect of IBA and different rooting media on lemon cultivar Pant Lemon-1 cuttings, Shira and Kumar (2015) found that soil and sand (1:1) medium had the maximum shoot fresh and dry weight per rooted cutting. Studied the effect of different rooting media and found that the maximum fresh and dry weight of shoots were observed with cutting planted in cocopeat, perlite and vermicompost (2:1:1) [28].

3.2. Root Growth and Development

3.2.1 Number of Roots Generated per Cuttings

The highest root number were observed in hardwood cuttings of mulberry planted in peat moss and sand (1:3) medium [10]. While evaluating the effect of different IBA concentrations and various media on rooting of fig (*Ficus carica*) cv. Black Ischia cuttings, observed that the number of roots were maximum in mixture of pond soil, river sand and vermicompost (2:1:1) [26]. The data of rooting response of *M. indica* variety V1 cuttings in rooting media and found that the cuttings planted in soil: FYM: Coir pith (1:1:1) had the highest

number of roots [24]. The rooting medium in a soil, red soil and vermicompost in ratio 2:1:1 on the rooting of dragon fruit showed better results in number of root per cutting [18]. While evaluating the effect of various suitable media on rooting of mulberry cv. Thar Harit cuttings, observed that the number of roots were maximum in mixture of cocopeat, perlite and vermicompost (2:1:1) [28].

3.2.2. Fresh and Dry Weight of Roots Per Cuttings

The rooting potential of schefflera stem cuttings planted in sand-peat moss (1:1) medium proved to be significantly superior with regard to fresh and dry weight of roots (Helaly et al. 2024). While evaluating the effect of different medium on rooting of hardwood cuttings of mulberry (*Morus alba* L.), Singh et al. (2015) found that the maximum fresh and dry root weight were recorded in soil, sand and FYM (1:1:1). The rooting medium in a soil, red soil and vermicompost in ratio 2:1:1 on the rooting of dragon fruit showed better results in fresh and dry weight of root [18]. While evaluating the effect of different medium on rooting of hardwood cuttings of mulberry (*Morus alba* L.), found that the maximum fresh and dry root weight were recorded in cocopeat, perlite and vermicompost (2:1:1) [28].

3.2.3. Root Diameter (mm)

The rooting potential of schefflera stem cuttings planted in cocopeat, perlite and vermicompost (2:1:1) medium proved to be significantly superior with regard to root diameter [28]. While studying the effect of types of rooting medium, stem cutting and growth regulator on rooting potential of schefflera, Helaly et al. (2024) observed that root diameter was significantly increased in cutting planted in sand-peat moss (1:1) medium. The effect of soil addition on physical properties of perlite-based media on strawberry cultivar Camarosa, it was found that root diameter was maximum on soil addition than control (100% perlite) [35]. The rooting medium in a soil, red soil and vermicompost in ratio 2:1:1 on the rooting of dragon fruit showed better results in root diameter [18].

3.2.4. Length of Longest Root (cm)

The root length was recorded maximum in the hardwood cuttings of mulberry with IBA @ 6000 ppm and planted in peat moss and sand ratio of 1:3 [10]. While studying the rooting response of cuttings of fig (*Ficus carica*) cv. Black Ischia as effected by rooting media, observed that the planted in pond soil, river sand and vermicompost had the highest root length [25]. The effect of different growing media on propagation of *M. indica* variety V1 cuttings and reported that root length was maximum in soil: sand: vermicompost (1:1:1) medium [54]. Evaluated the root growth of *Morus alba* as affected by types of cutting and rooting media and results showed that maximum root length was recorded under hardwood cutting planted in sand, FYM and clay (1:1:1) medium [43]. The rooting medium in a soil, red soil and vermicompost in ratio 2:1:1

on the rooting of dragon fruit showed better results in longest root length [18]. While studying the rooting response of cuttings of mulberry cv. Thar Harit as effected by rooting media, Observed that the planted in media combinations of cocopeat, perlite and vermicompost (2:1:1) had the highest root length [28]. Reported that the substrate mixture comprising cocopeat, perlite and vermicompost in a 3:1:1 ratio proved most effective for enhancing the strawberry root system [16]. Plants grow in this medium exhibited the greatest maximum root length and the highest total root length, indicating superior root development compared with other tested substrate combination. Vertical PVC column (90 mm) filled with soilless substrate mixture of cocopeat + poerlite + vermicompost (3:1:1) produced maximum root surface area, number of secondary roots/ primary root, average length of primary and secondary roots and per cent increase in the length of most developed root in strawberry cv Sweet Charlie [47].

3.2.5. Root Volume (ml)

The rooting medium in a soil, red soil and vermicompost in ratio 2:1:1 on the rooting of dragon fruit showed better results in root volume [18]. While studying the effect of growth regulator on rooting of semi-hardwood cuttings of pomegranate observed that root volume was significantly increased in hardwood cutting treated with higher concentration of IBA [56]. Observed the effect of different growing media on propagation of mulberry cuttings and reported that root volume was maximum in media combinations of cocopeat, perlite and vermicompost in ratio of 2:1:1 [28]. Reported that the substrate combination of cocopeat, perlite and vermicompost in a 3:1:1 ratio significantly enhanced root development, resulting in higher root density and root length density [13].

3.3. Physiological and Nutritional Response

3.3.1 Nutrients Status of Leaves

The effect of different growing media on propagation of mulberry cuttings and reported that nitrogen, phosphorus, potassium and chlorophyll content was maximum in media combinations of cocopeat, perlite and vermicompost in ratio of 2:1:1 [28].

3.3.2. Influence of Timing and Method

The review highlights examined the different planting dates (December 25, January 10 and January 25), three growth environmental conditions (open field, partial shade and mist chamber) was the affected in mulberry cutting propagation. According to [18, 19] reported that the hardwood stem cuttings, 15 cm long with 4–6 nodes, were taken from 5–6-year-old mulberry plants. Among the different treatments, the best results were observed when cuttings were planted on January 10 and grown in a mist chamber. Four local mulberry varieties Beyrudi, Hatuni, Sami and Yabani were also tested for survival rate and rooting success. Cuttings 20–25 cm long were

taken during the dormant season from one-year-old shoots (on January 15 in the first year and February 20 in the second year). These cuttings were dipped in IBA solutions of varying concentrations for 10 seconds. Rooting performance was evaluated 93–118 days later by measuring length of longest root, rooting percentage and the number of roots per cutting. The best rooting was achieved with IBA 5000 ppm, with 'Beyrudi' showing the highest rooting rate. According to Polat [37] reported that another experiment tested the effect of IBA and cinnamic acid (CA) on black mulberry cuttings in Tokat. Cuttings were collected in July, September, November and January and treated with 6000 ppm IBA, 6000 ppm IBA + 100 ppm CA, or 100 ppm CA alone. These were placed in perlite-filled, bottom-heated beds for 60 days. The highest rooting rate and average number of roots per cutting were recorded in cuttings taken in November and January treated with 6000 ppm IBA + 100 ppm CA [20].

4. Micro-Propagation

In vitro propagation of mulberry was carried out using axillary buds cultured on Murashige and Skoog (MS) medium. Shoot initiation was enhanced by supplementing the medium with different concentrations of 6-benzylaminopurine (BAP) along with 1 mg/L kinetin. The highest shoot initiation rate (90%) was observed with 2 mg/L BAP. When these initiated shoots were sub cultured on MS medium with 2 mg/L BAP and 1 mg/L kinetin, the average number of shoots increased. Maximum elongation (average of 19 elongated shoots) was recorded on MS medium containing 1 mg/L BAP and 1 mg/L kinetin. For root development, the best result (70% rooting rate) was obtained when elongated shoots were transferred to MS medium supplemented with 2 mg/L indole-3-butyric acid (IBA). These rooted plantlets, when hardened in peat moss pots in a greenhouse, showed an 80% survival rate [4]. The hardened plantlets, acclimated using a 1:1 mix of vermicompost and soil, exhibited a survival rate of approximately 70% under natural field conditions [44]. Apical buds of *Morus indica* L. were cultured on LSBM medium enriched with various concentrations of BAP and TIBA. The optimal medium for shoot initiation (94% response) and shoot multiplication (10.6 shoots per explant) was found to be LSBM supplemented with 8.88 μ M BAP and 2 μ M TIBA. These axenic plants, after gradual hardening, were successfully established in the field with a 90% survival rate [22]. In another study using *Morus alba* L., nodal explants and shoot tips cultured on MS medium with BAP and kinetin showed high sprouting rates 80% for nodal explants and 70% for shoot tips. Rapid in vitro proliferation was observed when explants were grown on MS medium containing 2 mg/L BAP and 0.2 mg/L NAA. For enhanced shoot multiplication, a combination of BAP (2 mg/L), NAA (0.2 mg/L), asparagine (25 mg/L) and glutamate (1 mg/L) was most effective. Around 80% of shoots grown on MS medium with 1.0 mg/L NAA successfully developed roots [2]. Field-grown mulberry explants were also cultured on MS

medium with different phytohormone combinations.

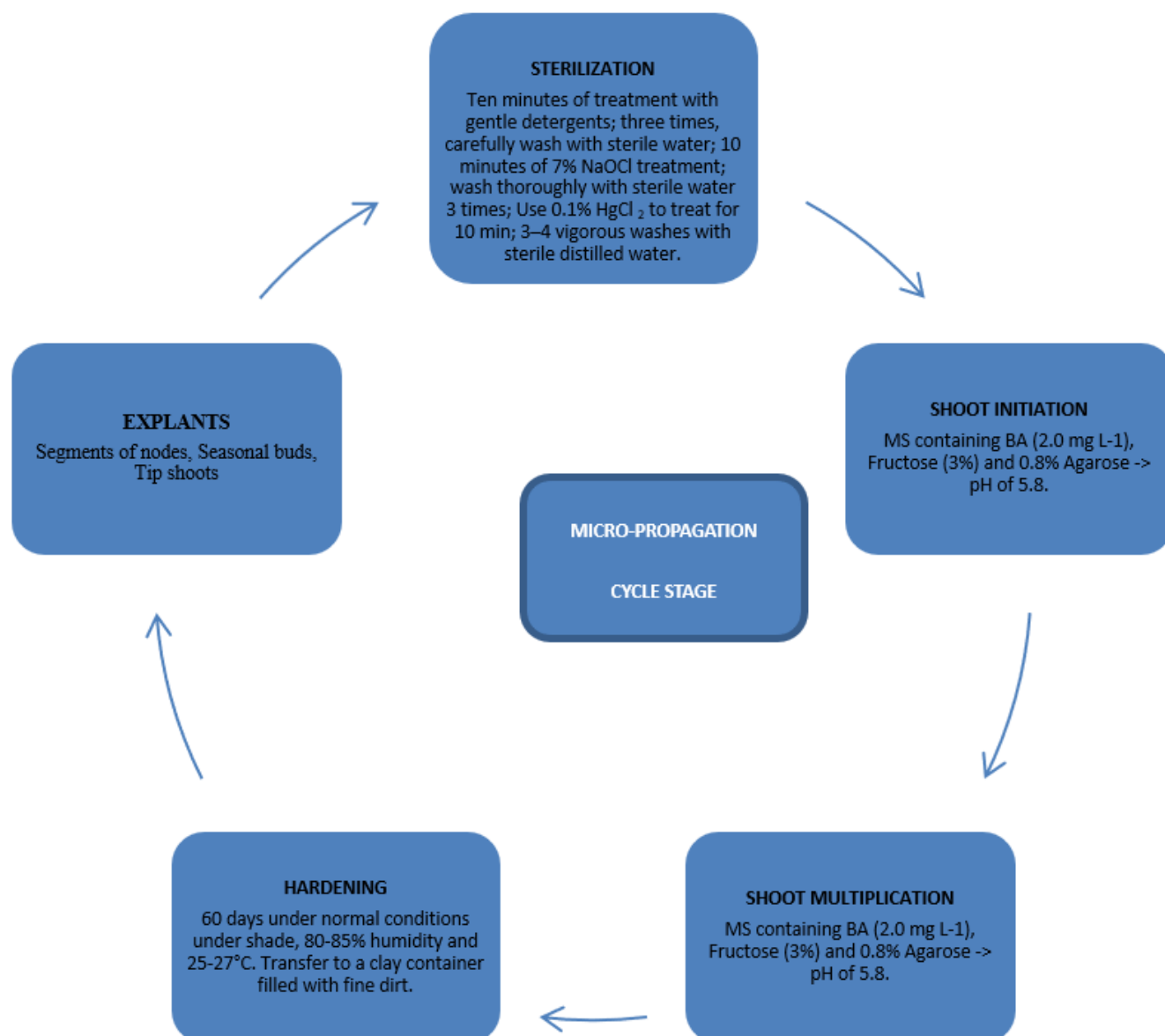


Figure 1. In vitro application of tissue culture techniques for rapid propagation and genetic improvement of mulberry (*Morus alba*) genotypes [40].

MS medium supplemented with BAP (1.0 mg/L), TDZ (0.1 mg/L) and NAA (0.25 mg/L) produced multiple shoots from nodal segments. Adventitious shoots proliferated on secondary medium containing BAP (1.0 mg/L), NAA (0.25 mg/L) and gibberellic acid (GA3, 0.5 mg/L). Root induction was successfully achieved using MS medium with 0.5 mg/L IBA, with or without 1% (w/v) charcoal. The resulting plantlets, when hardened, achieved a 98% survival rate under greenhouse conditions. Overall, these micropropagation techniques (Figure 1) are highly effective and hold great potential for both transgenic applications and large-scale multiplication of mulberry plants [40, 45].

5. Conclusion

The successful propagation of mulberry through cuttings can be significantly enhanced by the judicious use of plant growth regulators, appropriate rooting media and optimal timing. Indole-3-butyric acid (IBA) has proven to be particularly effective in improving survival rates, rooting percentages and root development; however, careful attention must be paid to its concentration, as excessive amounts may have inhibitory effects. Rooting media such as cocopeat, perlite, vermiculite and vermicompost offer considerable benefits by providing enhanced aeration, moisture retention and essential nutrients

uptake, all of which contribute to vigorous and healthy root systems. By strategically combining the right plant growth regulators with suitable rooting substrates, practitioners can achieve higher propagation success rates, ensuring the multiplication and preservation of desirable mulberry varieties. This integrated approach not only promotes more efficient cultivation practices but also holds great promise in increasing both the yield and quality of mulberry crops. Ultimately, such advancements can lead to greater profitability in sericulture and related industries, thereby improving the livelihoods of farmers and supporting the sustainable growth of the mulberry sector.

Abbreviations

BAP	6-Benzylaminopurine
CA	Cinnamic Acid
FYM	Farmyard Manure
GA ₃	Gibberellic Acid
IBA	Indole-3-Butyric Acid
LSBM	Low Salt Basal Medium
MS	Murashige and Skoog Medium
NAA	Naphthalene Acetic Acid
PGRs	Plant Growth Regulators
TDZ	Thidiazuron
TIBA	2,3,5-Triiodobenzoic Acid

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Conflicts of Interest

The author declares no conflict of interest.

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