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A comprehensive review of mulberry (*Morus* spp.) propagation: From conventional methods to biotechnological innovations

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Abstract
Mulberry (*Morus* spp.) is an economically important perennial crop cultivated mainly for sericulture, where the quality and availability of planting material directly influence productivity. Since seed propagation results in genetic variability and delayed establishment, vegetative propagation methods are widely used for maintaining superior genotypes and producing uniform plants. This review summarizes conventional and advanced mulberry propagation techniques, including stem cutting, grafting, budding, layering, root segment multiplication, and mini-clonal technology. Stem cuttings remain the most common method due to their simplicity and cost-effectiveness, while rooting success is influenced by genotype, physiological condition of cuttings, environmental factors, and application of growth regulators such as indole-3-butyric acid (IBA). Grafting, budding, and layering provide alternative approaches for improving propagation efficiency and conserving valuable cultivars, whereas micropropagation and mini-clonal technologies offer rapid multiplication of genetically uniform planting materials under controlled conditions. The integration of traditional and biotechnological approaches can enhance mulberry improvement programmes by ensuring the availability of high-quality planting material, supporting germplasm conservation, and promoting sustainable sericulture production.
This review highlights the principles, applications, advantages, and limitations of different mulberry propagation strategies and emphasizes the need for further research on physiological, molecular, and environmental factors influencing regeneration and establishment. A combined approach involving traditional propagation practices and advanced biotechnological tools will be crucial for future improvement and expansion of mulberry-based production systems.

Keywords: Mulberry (*Morus* spp.), vegetative propagation, stem cutting, grafting, budding, layering, micropropagation, mini-clonal technology, sericulture

Introduction
Poor adventitious root formation is a significant hurdle in micropropagation and in conventional propagation of mulberry plants. These root systems are vital to carbon sequestration, nutrient cycling, and the hydrologic cycle. Furthermore, rooting depth serves as a critical variable in general circulation models, as it helps regulate evapotranspiration. Roots essentially bridge the gap between groundwater supplies, stream flows, and transpiration demands and root formation is a crucial parameter for all plantations. Additionally, the physical and biochemical conditions created by roots are closely linked to soil microorganisms and nutrients. Because root formations are sensitive to changes in climate and land use, they remain a central element in plant propagation (Jobbágy and Jackson, 2004; Schenk and Jackson, 2002b). Comprehensive insights into adventitious rooting as a developmental progression, emphasizing the influence of endogenous growth regulators and the impact of environmental substrates on subsequent vegetative vigor remains a pivotal study in mulberry plantations [39, 40].
The commercial multiplication of mulberry typically utilizes hardwood cuttings, a method favored for its capacity for rapid progeny expansion and the preservation of specific genetic traits. However, the temporal window for preparing these cuttings serves as a fundamental determinant in rooting efficacy, as success is intrinsically linked to

the physiological state of the donor plant and ambient ecological factors. Rooting outcomes demonstrate significant variability based on seasonal shifts and localized thermal regimes. Furthermore, the regenerative potential of stem segments is governed by a complex interplay of variables, including genotype-specific requirements, the topographical origin and maturity of the cutting, and the nutritional status of the stock material. Additional influences such as mechanical wounding, etiolation, hormonal applications, and the physical properties of the growth substrate also modulate developmental success (Hartmann *et al.*, 2002). As noted by Harrison-Murray (1991), the phenological timing of collection remains a decisive factor in the rooting process. Within the context of plant propagation, the diverse propagation environments, including glass houses, net houses, polyhouses, and mist chambers, are extensively utilized to facilitate rooting across various cutting categories. The advent of mist chamber technology represents a seminal advancement in botanical propagation. Research by Khan *et al.* (2007) evaluated the developmental performance of Morus alba L. cuttings within open-air conditions versus polythene low tunnels. Their findings indicated that while maximum sprouting and foliar development were observed in eight-inch segments, vertical growth peaked in six-inch cuttings; notably, these metrics were significantly enhanced within the polythene tunnel environment. Furthermore, Kalyoncu *et al.* (2009) identified optimal rooting efficiency in black mulberry through the application of 2000 and 3000 ppm IBA concentrations, achieving full success. Comparative analyses by Singh *et al.* (2014) corroborating these results demonstrated that treatments utilizing 2000 mg.L-1 of IBA yielded superior outcomes regarding sprout count, root elongation, and overall rooting frequency [48, 13, 41, 42, 433].

A multitude of academic investigations have elucidated the formidable obstacles inherent in the propagation of Morus species, wherein suboptimal adventitious rooting efficiency presents a substantial barrier to effective cultivation Qiu *et al.*, 2005]. Although the refinement of agricultural practices has demonstrated efficacy in improving rooting metrics, a demand for standardizing agricultural methodologies for maximizing seedling/cutting survivability persists. The physiological vigor of these plants is primarily dependent on their rooting such as absorption and translocation of vital nutrients [Sourati *et al.*, 2022], rendering them highly vulnerable to adverse soil conditions that can impede growth and development, even jeopardizing the survival of mulberry seedlings and may restrict developmental progress or compromise plant viability. Consequently, optimizing the physiological parameters governing mulberry seedling growth, as well as fortifying root capabilities in soil moisture and nutrient absorption, can highly improve upon mulberry growth. The utilization of cutting methodologies has demonstrated potential in enhancing the survivability of mulberry specimens. The strategic use of plant growth regulators can accelerate essential developmental stages including cell division, rhizome extension, and the maturation of flowers and fruit specifically for tree species that are famously difficult to root. The judicious application of plant growth regulators can expedite crucial processes such as cell division, rhizome elongation, and floral and fruit maturation [27, 32].

Mulberry cultivation for fruit production necessitates clonal propagation, typically utilizing grafting or cutting techniques rather than tissue culture or layering. Hardwood cuttings represent one of the most accessible and cost-effective strategies for vegetative multiplication. Recent advancements have highlighted the efficacy of auxin treatments in stimulating root development in these cuttings.

The success of adventitious rooting in stem cuttings is governed by several critical factors: genotype-specific requirements, the topographical origin and type of cutting, physiological preparation such as wounding or etiolation, and the timing of collection. Furthermore, environmental and supplemental conditions including growth media, misting, bottom heat, hormonal applications, and nutritional enrichment significantly influence rooting outcomes.Indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are the primary root-promoting chemicals used in nurseries due to their low toxicity across varied concentrations. IBA, in particular, is vital for both softwood and hardwood segments. Research indicates that concentrations of 2000 ppm to 3000 ppm of IBA yield optimal results, maximizing sprouting frequency, root elongation, and overall rooting percentages. The propagation substrate is an essential component of the growth system, directly impacting root proliferation. Recommended media often include peat or specialized mixtures such as peat, perlite, and cocopeat in a 2:2:1 ratio to enhance plant height and foliar development. Other effective combinations include perlite paired with peat, vermiculite, or coconut fiber. Mixtures of perlite and vermiculite have traditionally been used in Californian nurseries (Sardoei, 2014). Kako AlZebari and Ali M. Al-Brifkany (2015) observed that the Media (1 part peat moss + 2 parts sand) increased rooting percentage in the stem cuttings of (Citrus medica Linnaeus) Corsian cultivar. In mulberry research, hardwood cuttings have been extensively studied, yet investigations into softwood cuttings remain sparse and frequently produce inconsistent findings. Moreover, the targeted use of exogenous plant growth regulators could serve a dual purpose: it significantly improves rooting efficiency and can adjust stress resilience by triggering internal hormonal systems, with the specific concentration of the application determining the subsequent oxidase activity [32, 45].

Global Trends in Mulberry Propagation

Mulberry (*Morus* spp.) is cultivated globally utilizing both sexual (seed-based) and asexual (vegetative) techniques. The selection of a particular propagation strategy is influenced by factors such as the production scale, material availability, specific genotype, and the ultimate goals of cultivation. Within commercial sericulture, vegetative methods are predominantly favored as they ensure the maintenance of genetic uniformity and the conservation of elite characteristics, including disease resistance, leaf quality, and yield.

Seed Propagation in Mulberry

Seed propagation is one of the oldest and most fundamental methods of mulberry (*Morus* spp.) multiplication. Historically, it has played a crucial role in the domestication, genetic improvement, and geographical distribution of mulberry species throughout Asia, Europe, and North America. In this method, plants are raised from seeds obtained from mature fruits, resulting in seedlings that possess a unique combination of parental genetic characteristics. Although seed propagation is relatively simple and inexpensive, its use in commercial mulberry cultivation is limited because mulberry is a highly heterozygous and predominantly cross-pollinated species, leading to considerable genetic variability among seed-derived progenies (Vijayan *et al.*, 2011).

The primary advantage of seed propagation lies in its ability to generate genetic diversity, which serves as the foundation for breeding and selection programs. Seedlings provide a valuable source of novel genetic combinations that can be screened for desirable traits such as high leaf yield, improved nutritional quality, disease resistance, drought tolerance, and adaptability to diverse environmental conditions. Consequently, seed propagation remains an indispensable tool in mulberry improvement programs and germplasm conservation efforts worldwide (Datta, 2000). In addition, seedlings are frequently used as rootstocks for grafting and budding, particularly in regions where vegetative propagation is difficult or where vigorous root systems are required (Tikader and Vijayan, 2010).

Successful seed propagation depends on seed quality, viability, dormancy status, and environmental conditions during germination. Mulberry seeds generally exhibit moderate to high germination potential when collected from fully ripe fruits and subjected to appropriate pre-sowing treatments. Cold stratification, soaking in water, and treatment with growth regulators such as gibberellic acid (GA_3) have been reported to improve germination percentage and seedling vigor in several mulberry species (Yadav *et al.*, 2014). Germination is usually epigeal, with seedlings establishing rapidly under favorable moisture and temperature conditions. However, considerable variation in growth rate, morphology, leaf characteristics, and adaptability is commonly observed among seedlings due to their genetic heterogeneity.

Despite its significance in breeding and conservation, seed propagation possesses several limitations for commercial mulberry cultivation. The major drawback is the inability to maintain genetic uniformity, resulting in plantations with variable growth habits, leaf yield, and quality. Such variability is undesirable in sericulture, where consistent leaf production is essential for silkworm rearing. Furthermore, seed-propagated plants often require a longer juvenile phase before attaining full productivity compared with vegetatively propagated plants. Consequently, stem cuttings, grafting, and tissue culture techniques have largely replaced seed propagation for large-scale commercial multiplication of elite mulberry cultivars (Machii *et al.*, 2002).

Recent advances in seed biology and molecular genetics have enhanced the utilization of seed propagation in mulberry improvement programs. Molecular markers and genomic tools are increasingly employed to characterize seedling populations, identify superior genotypes, and accelerate breeding efforts. Moreover, seed-based conservation remains an important strategy for preserving the genetic diversity of wild and cultivated mulberry species, ensuring the availability of valuable genetic resources for future crop improvement and adaptation to changing environmental conditions (Vijayan *et al.*, 2011) [37, 21, 10, 34, 38, 21].

Overall, seed propagation continues to serve as a vital method for genetic enhancement, rootstock production, and germplasm conservation in mulberry. Although it has limited application in commercial clonal multiplication, its contribution to breeding programs and the development of improved cultivars remains indispensable for the long-term sustainability of mulberry cultivation.

Mulberry Multiplication via Hardwood Cuttings: A Vital Stem Propagation Technique

Among the various vegetative techniques, the use of hardwood cuttings is recognized as one of the most prevalent strategies for multiplying *Morus* species, particularly in commercial sericulture because it enables rapid multiplication of elite cultivars while maintaining the desirable characteristics of the mother plant. Since mulberry is highly heterozygous and seed propagation often results in variability, vegetative methods such as stem cuttings are preferred for establishing uniform plantations. Stem cuttings also reduce the juvenile period compared with seed-grown plants and allow large-scale production of planting material.

In mulberry propagation, hardwood cuttings obtained from healthy, mature shoots are commonly used. Suitable cuttings are generally collected from vigorous one-year-old shoots, with adequate diameter and several healthy buds. The basal end of the cutting is placed in a well-drained rooting medium, where adventitious roots develop from the stem tissues. Factors such as genotype, physiological age of the cutting, season of collection, moisture availability, rooting medium, and application of plant growth regulators strongly influence rooting success. Recent studies have shown that the type of growing medium and concentration of indole-3-butyric acid (IBA) significantly affect root and shoot development in mulberry cuttings.

Auxins such as IBA and naphthalene acetic acid (NAA) are frequently applied to improve rooting efficiency. These hormones stimulate cell division and differentiation at the basal portion of the cutting, promoting faster initiation and development of adventitious roots. Singh *et al.* reported that mulberry stem cuttings treated with different concentrations of IBA and NAA under mist-house conditions showed improved rooting characteristics, with higher rooting percentage and better root growth observed with IBA treatment, particularly at 2000 mg L^{-1}. Similarly, studies on *Morus alba* have demonstrated that appropriate concentrations of IBA and NAA enhance root number, sprouting, and overall establishment of cuttings.

Environmental conditions after planting are critical for successful rooting. High humidity, moderate temperature, and adequate but not excessive moisture help prevent dehydration of cuttings before root formation. Mist chambers and protected nursery conditions are often used because they maintain favourable humidity and reduce water loss. Research on mulberry has also investigated the physiological and molecular processes involved in adventitious root formation, highlighting that successful rooting depends on complex interactions between hormones, carbohydrate reserves, and gene regulation during early root development.

Stem cutting propagation offers several advantages for mulberry cultivation, including rapid multiplication, uniformity of plantations, early establishment, and preservation of superior genetic traits. However, rooting ability varies among mulberry species and cultivars, and some genotypes show poor adventitious root formation. Optimizing cutting type, hormone treatment, rooting substrate, and nursery management practices is therefore essential for efficient commercial propagation.

Globally, particularly across Asia in nations like India, China, and Bangladesh, stem cutting propagation stands as the primary technique for mulberry multiplication. This approach utilizes hardwood, semi-hardwood, or softwood segments depending on specific species requirements and local ecological factors. The widespread preference for this method stems from its cost-effectiveness, simplicity, and speed, alongside its ability to maintain genetic consistency by producing true-to-type offspring. The development of adventitious roots typically occurs at the stem nodes, a process that can be further stimulated by applying auxins such as NAA and IBA. Due to its straightforward nursery management and high efficiency, stem cuttings continue to be the fundamental propagation strategy for numerous commercial mulberry cultivars.

Vegetative propagation through hardwood cuttings is one of the most widely adopted methods for the multiplication of mulberry (*Morus* spp.), particularly in commercial sericulture systems where genetic uniformity and rapid establishment of plantations are essential. Compared with seed propagation, hardwood cuttings preserve the desirable traits of elite cultivars and ensure true-to-type progenies. The method is simple, economical, and suitable for large-scale nursery production, making it the preferred propagation technique in many mulberry-growing regions. Studies have shown that hardwood cuttings obtained from mature, healthy shoots can produce satisfactory rooting and establishment when environmental conditions and management practices are optimized (Shravankumar *et al.*, 2023).

The success of hardwood cutting propagation in mulberry largely depends on factors such as cultivar, physiological age of the mother plant, season of collection, rooting medium, and the application of plant growth regulators. Among these factors, indole-3-butyric acid (IBA) has received considerable attention for enhancing adventitious root formation. Several investigations have demonstrated that treatment of hardwood cuttings with IBA concentrations ranging from 1,500 to 6,000 ppm significantly improves rooting percentage, root number, and root length. For example, rooting success of up to 66.7% was reported in mulberry hardwood cuttings treated with 1,500 ppm IBA, whereas untreated cuttings exhibited substantially lower rooting percentages (Shravankumar *et al.*, 2023). Similarly, Aziz *et al.* (2024) observed improved rooting in black mulberry (*Morus nigra*) when hardwood cuttings were soaked in water prior to treatment with 4,000 ppm IBA, indicating the importance of both hydration and hormonal stimulation in root induction [30, 2].

Despite its practical advantages, hardwood cutting propagation in mulberry is often constrained by genotype-dependent rooting ability. Some species and cultivars exhibit poor rooting due to limited adventitious root formation, resulting in lower propagation efficiency. Physiological and molecular studies have revealed that root initiation in hardwood cuttings involves complex hormonal regulation, carbohydrate mobilization, and gene expression changes associated with root primordia development. Advances in understanding these mechanisms have led to improved propagation protocols capable of achieving rooting percentages approaching 90% under controlled conditions (Jiao *et al.*, 2019) [17]. Furthermore, innovations such as mist chambers, aeroponic systems, and controlled temperature-humidity environments have enhanced rooting performance and transplant survival of mulberry cuttings.

Overall, hardwood cuttings remain a reliable and cost-effective method for mulberry propagation. Continued refinement of propagation techniques, particularly through the optimization of growth regulator treatments and environmental conditions, will further improve rooting success and support the large-scale production of high-quality planting material for sericulture and horticultural purposes.

Techniques for Grafting *Morus* Species

Grafting serves as a vital vegetative multiplication strategy in the cultivation and genetic enhancement of mulberry. This approach involves the integration of a scion, which is a chosen shoot segment from a high-quality cultivar, with a suitable rootstock to form a unified, growing plant. Such a methodology is particularly effective for propagating elite genotypes and enhancing overall plant robustness. Additionally, grafting provides a solution for cultivars that exhibit poor natural rooting and allows for the development of specimens with specific advantageous characteristics, including superior leaf productivity, resistance to pathogens, and resilience to varying environmental stressors.

Grafting is an important vegetative propagation technique used in mulberry (*Morus* spp.) improvement and cultivation, particularly for combining desirable characteristics of two different plants. In grafting, a selected shoot portion (scion) from a superior cultivar is joined with a compatible rootstock so that the two parts unite and develop as a single plant. The method is useful for multiplying elite genotypes, improving plant vigour, overcoming problems associated with poor rooting, and producing plants with desirable traits such as high leaf yield, disease tolerance, and adaptability to environmental conditions (Dandin and Giridhar, 2014) [8].

Several grafting methods have been attempted in mulberry, including cleft grafting, wedge grafting, whip-and-tongue grafting, side grafting, and approach grafting. Among these, cleft and wedge grafting are commonly practiced because they provide better cambial contact and higher graft success rates. In cleft grafting, a vertical split is made in the rootstock or stem, and a wedge-shaped scion containing healthy buds is inserted into the split. Proper alignment of the cambium layers of the scion and stock is essential for successful union formation. In whip-and-tongue grafting, both stock and scion are cut with matching sloping surfaces and interlocked through tongue cuts, providing greater surface contact and mechanical stability.

The success of grafting in mulberry depends on several factors, including the choice of rootstock and scion, physiological age of plant material, season of grafting, environmental conditions, and aftercare practices. Actively growing rootstocks with vigorous cambial activity generally promote faster callus formation and vascular connection between stock and scion. The best grafting period is usually when temperature and moisture conditions favour active growth, as rapid callus formation improves graft union development. Protection of the grafted region with grafting tape or suitable wrapping materials helps maintain moisture and prevents infection during the healing process (Tikader and Vijayan, 2011) [35].

Grafting is also used in mulberry breeding programmes and conservation of valuable germplasm. It enables the maintenance of difficult-to-root cultivars and facilitates evaluation of superior genotypes on uniform root systems. Studies have demonstrated that graft compatibility, anatomical connection between vascular tissues, and hormonal interactions play significant roles in determining graft survival and subsequent plant growth. Successful graft unions involve the formation of callus tissue, differentiation of new vascular tissues, and establishment of functional xylem and phloem connections between stock and scion (Melnyk and Meyerowitz, 2015) [23].

Although grafting provides several advantages, its large-scale adoption in mulberry is limited compared with stem cutting propagation because it is more labour-intensive and requires skilled workers. Variations in graft compatibility among cultivars and the need for careful management during the healing phase are additional constraints. Nevertheless, grafting remains a valuable technique for improving mulberry productivity, developing superior planting materials, and supporting breeding and research programmes.

Budding Techniques in Mulberry (*Morus* spp.)

As a specialized subset of grafting, budding entails the insertion of a solitary vegetative bud from a target cultivar into a donor rootstock. While T-budding and patch budding are less ubiquitous than stem cuttings, they facilitate the rapid expansion of elite clones while requiring minimal scion material. When conducted under optimized ecological parameters, these protocols demonstrate high efficiency and remains a vital component within specialized nursery and botanical breeding programs (Shravankumar *et al.*, 2023) [30].

Budding is a vegetative propagation technique in which a single vegetative bud from a desirable mulberry (*Morus* spp.) cultivar is inserted onto a compatible rootstock to develop into a new plant. Unlike grafting, where a portion of the stem containing several buds is used as the scion, budding utilizes only a single bud along with a small portion of surrounding bark and cambium tissue. This method is useful for rapid multiplication of elite mulberry varieties, maintenance of superior genetic traits, and production of uniform planting material for sericulture (Dandin and Giridhar, 2014) [8].

Several budding methods have been studied and practiced in mulberry, including shield budding (T-budding), patch budding, chip budding, and ring budding. Among these, T-budding and patch budding are the most commonly used techniques. In T-budding, a T-shaped incision is made on the bark of the rootstock, and a shield-shaped bud piece containing a healthy axillary bud is inserted beneath the bark flaps. The union is then wrapped with budding tape to maintain close contact between the bud and stock tissues. Patch budding involves removal of a rectangular patch of bark containing a bud from the scion and replacing it with a similar patch removed from the rootstock. This method provides a larger cambial contact area and is often preferred when the bark separates easily from the wood.

The success of budding in mulberry depends on factors such as the age and vigour of the rootstock, maturity of the budwood, environmental conditions, and compatibility between stock and bud. Actively growing plants with good cambial activity favour successful bud union because the cambium produces callus tissue that connects the vascular systems of the two tissues. Adequate moisture, moderate temperature, and proper protection of the budding site are essential for preventing desiccation and infection during the healing process (Hartmann *et al.*, 2014) [14].

Budding has several advantages in mulberry propagation, including the requirement of less scion material, rapid multiplication of valuable cultivars, and the possibility of using vigorous rootstocks to improve plant establishment. It is particularly useful for conserving rare or superior genotypes where limited propagation material is available. Budding can also be used in breeding programmes for evaluating promising selections and combining desirable characteristics of different plant materials.

However, budding in mulberry may be affected by cultivar differences, seasonal limitations, and variation in bud take percentage. Proper selection of mature but healthy buds, skilled handling, and suitable timing are necessary for obtaining high success rates. Although stem cutting remains the dominant commercial propagation method in many sericulture regions, budding continues to be an important supplementary technique for mulberry improvement, germplasm conservation, and specialized nursery production.

Layering Strategies

Layering facilitates root induction while the developing segment maintains physiological attachment to the mother plant. Diverse modalities such as simple, mound (stooling), trench, and air layering (marcotting) are utilized, with air layering being particularly efficacious for recalcitrant species like *Morus nigra*. Because the propagule benefits from continuous hydration and nutrient translocation from the parent, this method often yields superior establishment rates compared to detached segments, though its labor-intensive nature limits its application in large-scale commercial multiplication (Agriculture Institute, 2025) [1].

Layering is a vegetative propagation method in which roots are induced on a stem while it remains attached to the parent plant. After sufficient root development, the rooted portion is separated and established as an independent plant. In mulberry (*Morus* spp.), layering is used as an alternative propagation technique, particularly for multiplying difficult-to-root cultivars, conserving superior genotypes, and producing vigorous planting materials with a high survival rate. Since the newly formed plant remains connected to the mother plant during root initiation, it receives continuous water and nutrient supply, which improves establishment compared with detached cuttings (Hartmann *et al.*, 2014) [14].

Different forms of layering have been reported in mulberry, including simple layering, mound layering (stooling), air layering (marcotting), and serpentine layering. In simple layering, a low-growing flexible branch is bent toward the soil, a portion of the stem is wounded or covered with soil, and the exposed tip is allowed to grow vertically. Adventitious roots develop from the buried section, and the rooted layer is later detached from the mother plant. Mound layering involves cutting back the parent plant and covering the emerging shoots with moist soil to encourage root formation at the shoot bases. This technique is particularly useful for producing multiple rooted shoots from a single plant.

Air layering is another useful method in mulberry, especially when suitable low branches are unavailable or when propagation material is limited. In this method, a selected branch is wounded by removing a ring of bark or making a suitable incision, and the wounded region is wrapped with a moist rooting medium such as moss, cocopeat, or soil mixture. The wrapped portion is maintained under humid conditions until roots develop, after which the rooted branch is detached and planted separately. Application of auxins such as indole-3-butyric acid (IBA) may improve root initiation and accelerate the layering process.

The success of layering in mulberry depends on several factors, including the physiological condition of the branch, season of operation, moisture availability, rooting medium, and environmental conditions. Actively growing shoots with sufficient carbohydrate reserves generally show better rooting response. Proper maintenance of humidity around the rooting zone is essential because dehydration can reduce root formation and survival. The use of growth regulators, particularly auxins, has been reported to enhance adventitious root development by stimulating cell division and differentiation at the wounded region (Hartmann *et al.*, 2014) [14].

Layering offers several advantages in mulberry propagation, including high survival percentage, production of true-to-type plants, and reduced dependence on specialized nursery facilities. It is particularly valuable for maintaining rare cultivars and genotypes that exhibit poor rooting ability through conventional cutting methods. However, layering is generally slower and less suitable for large-scale commercial multiplication compared with stem cutting propagation because each parent plant produces a limited number of propagules. Therefore, layering is mainly used as a supplementary propagation method in mulberry improvement programmes, germplasm conservation, and small-scale nursery production.

Root Segment Multiplication in Mulberry (*Morus* spp.)

Certain mulberry species possess the regenerative capacity to be multiplied via root segments capable of differentiating into adventitious shoots and roots. This approach serves as a critical alternative when aerial stem material is scarce or when specific genotypes exhibit poor response to conventional stem-cutting protocols. Although root cuttings are not the standard in commercial sericulture nurseries, they are recognized as a viable auxiliary method across several regional mulberry cultivation systems.

Root segment multiplication is a vegetative propagation technique in which portions of roots from a healthy mother plant are used to generate new shoots and subsequently develop into independent plants. This method is based on the ability of certain mulberry (*Morus* spp.) genotypes to produce adventitious buds or shoots from root tissues under favourable environmental conditions. Root propagation is particularly useful for conserving valuable genotypes, multiplying selected cultivars, and regenerating plants when conventional propagation materials such as stem cuttings or scions are limited.

In this method, healthy and disease-free roots are collected from vigorous donor plants, generally during the dormant or less active growth period when carbohydrate reserves are high. The roots are cut into suitable segments, usually containing adequate tissue for regeneration, and planted horizontally or vertically in a well-prepared rooting medium. Under favourable conditions of moisture, aeration, and temperature, the root segments produce callus tissue followed by adventitious shoot formation. Once shoots become sufficiently established, they are separated and transplanted for further growth.

The regenerative ability of root segments in mulberry is influenced by several factors, including genotype, root diameter, physiological age of the root, season of collection, environmental conditions, and hormonal balance. Young, healthy roots with active meristematic tissues generally show greater regenerative capacity. The application of plant growth regulators, particularly cytokinins for shoot induction and auxins for root development, may enhance regeneration efficiency by promoting cell division and organ differentiation. The balance between endogenous hormones and stored carbohydrates plays an important role in determining the success of root segment propagation.

Root segment multiplication provides certain advantages over conventional propagation methods. It can be used for multiplying cultivars with poor rooting ability from stem cuttings and offers an alternative source of planting material when shoot availability is limited. The technique also helps in maintaining genetic uniformity because plants regenerated from root tissues retain the characteristics of the parent plant. In addition, root-derived regeneration has potential applications in mulberry improvement programmes, germplasm conservation, and nursery multiplication.

However, root segment propagation is not widely adopted for commercial mulberry production because regeneration capacity varies considerably among cultivars and the number of propagules produced is generally lower than that obtained through stem cuttings. Careful selection of suitable root material and optimization of environmental and hormonal conditions are required to achieve reliable multiplication. Despite these limitations, root segment multiplication remains a valuable supplementary method for specialized propagation and conservation of elite mulberry genetic resources.

Application of Exogenous Plant Hormones to Enhance Rooting in Mulberry Cuttings

The utilization of exogenous plant growth regulators can modulate plant stress tolerance by triggering the activity of endogenous hormones. Furthermore, the reaction of root-associated oxidase activity to these regulators is contingent upon the specific hormonal concentrations applied. (Sun, 2023). The application of exogenous plant growth regulators, particularly auxins, is a widely recognized practice for improving adventitious root formation in mulberry (*Morus* spp.) cuttings. Rooting in stem cuttings is a complex physiological process regulated by endogenous hormones, carbohydrates, and environmental factors. However, natural auxin levels in hardwood cuttings are often insufficient to induce rapid and uniform root development, especially in difficult-to-root cultivars. Therefore, external application of auxins has become an effective strategy to enhance rooting percentage, root quality, and subsequent plant establishment.

Among the various plant growth regulators evaluated, indole-3-butyric acid (IBA) has been reported as the most effective auxin for inducing rooting in mulberry cuttings. IBA promotes cell division and differentiation in the cambial region, leading to the formation of root primordia and increased adventitious root emergence. Numerous studies have demonstrated significant improvements in rooting success following IBA treatment. Depending on cultivar, cutting type, and environmental conditions, concentrations ranging from 1,000 to 6,000 ppm have been found effective. Treatment with IBA not only increases the percentage of rooted cuttings but also enhances root number, root length, and root biomass, resulting in more vigorous nursery plants.

Other auxins such as naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) have also been investigated for their rooting-promoting effects. Although both compounds can stimulate root initiation, IBA is generally preferred because of its greater stability, lower susceptibility to degradation, and more consistent performance across different mulberry genotypes. In some studies, combinations of IBA and NAA have produced synergistic effects, further improving rooting characteristics compared with individual hormone treatments. Such combinations may enhance both root initiation and subsequent root elongation, thereby improving the overall quality of propagated plants.

The effectiveness of exogenous auxin application is influenced by the method of treatment, duration of exposure, and physiological condition of the cuttings. Quick-dip treatments using concentrated auxin solutions and prolonged soaking in dilute solutions are commonly employed in commercial nurseries. Environmental factors such as temperature, humidity, light intensity, and rooting substrate also interact with hormonal treatments to determine rooting success. Under favorable conditions, auxin-treated hardwood cuttings exhibit faster root initiation and higher survival rates after transplanting than untreated controls.

Recent physiological and molecular studies have shown that exogenous auxins regulate the expression of genes associated with root primordium formation, cell wall modification, and carbohydrate metabolism. These hormonal signals interact with endogenous pathways to stimulate the development of adventitious roots. Consequently, the application of plant growth regulators remains an essential component of modern mulberry propagation protocols, particularly for elite cultivars where rapid multiplication and uniformity are required. Continued research on hormone combinations, optimal concentrations, and their interaction with environmental factors may further enhance rooting efficiency and nursery production of mulberry planting material.

Utilizing Plant Growth-Promoting Bacteria (PGPBs) to Improve Rooting in Mulberry Cuttings

Plant growth-promoting bacteria (PGPBs) have emerged as a sustainable and environmentally friendly alternative to synthetic growth regulators for improving rooting and establishment of vegetatively propagated plants. These beneficial rhizobacteria colonize plant tissues and the rhizosphere, enhancing plant growth through various direct and indirect mechanisms. In mulberry (*Morus* spp.), the use of PGPBs has gained increasing attention due to their potential to stimulate adventitious root formation, improve nutrient uptake, and enhance survival of rooted cuttings under nursery and field conditions.

One of the principal mechanisms through which PGPBs promote rooting is the production of phytohormones, particularly auxins such as indole-3-acetic acid (IAA). Bacterial genera including *Azospirillum*, *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Enterobacter* are known to synthesize IAA, which stimulates cell division and differentiation in stem tissues, leading to the initiation and development of adventitious roots. In addition to auxin production, these microorganisms can influence endogenous hormonal balance by modulating ethylene levels through the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, thereby reducing stress-induced inhibition of root growth. As a result, cuttings treated with PGPBs often exhibit increased rooting percentage, greater root number, and enhanced root length compared with untreated controls.

Beyond hormonal effects, PGPBs contribute to rooting success through improved nutrient availability and uptake. Many bacterial strains possess the ability to solubilize phosphorus, fix atmospheric nitrogen, and produce siderophores that enhance iron acquisition. These activities improve the nutritional status of cuttings during the critical rooting phase, supporting vigorous root and shoot development. Furthermore, certain PGPBs produce extracellular enzymes and bioactive metabolites that facilitate root emergence and strengthen plant resistance to environmental stresses. Enhanced nutrient acquisition and stress tolerance collectively improve the quality and survivability of rooted mulberry cuttings.

Several studies on woody and horticultural crops have demonstrated positive interactions between PGPBs and exogenous auxins, suggesting that integrated use of beneficial bacteria and plant growth regulators can further enhance rooting performance. Although research specifically focused on mulberry is relatively limited, available evidence indicates that inoculation with beneficial bacterial strains can improve nursery establishment and plant growth. The application of microbial biofertilizers containing *Azotobacter*, *Azospirillum*, and phosphate-solubilizing bacteria has been reported to enhance root development and biomass accumulation in mulberry plants, supporting their potential role in vegetative propagation systems.

Recent advances in plant-microbe interaction research have highlighted the ability of PGPBs to influence the expression of genes associated with root initiation, nutrient transport, and stress responses. These findings provide a physiological and molecular basis for the observed improvements in rooting and plant vigor. Consequently, the incorporation of PGPBs into mulberry propagation protocols represents a promising strategy for reducing dependence on synthetic chemicals while promoting sustainable nursery production. Future research should focus on identifying effective bacterial strains, optimizing inoculation methods, and evaluating synergistic interactions between microbial inoculants and conventional rooting hormones to maximize propagation efficiency.

Tissue Culture as an Advanced Method of Adventitious Rooting in Mulberry

Tissue culture, also known as micropropagation, represents an advanced and highly efficient method for inducing adventitious rooting in mulberry (*Morus* spp.). Unlike conventional propagation techniques, tissue culture enables the rapid multiplication of elite genotypes under controlled aseptic conditions, ensuring the production of genetically uniform and disease-free planting material. The technique is particularly valuable for the propagation of superior cultivars that exhibit poor rooting ability through conventional stem cuttings and for the conservation of valuable germplasm (Thomas, 2002) [33].

Adventitious root formation in tissue culture is generally achieved through the manipulation of plant growth regulators in the culture medium. Auxins, particularly indole-3-butyric acid (IBA), α-naphthaleneacetic acid (NAA), and indole-3-acetic acid (IAA), play a crucial role in root induction and development. Microshoots regenerated from nodal segments, shoot tips, or axillary buds are transferred to rooting media supplemented with appropriate concentrations of auxins, which stimulate cell dedifferentiation and root primordia formation. Numerous studies have reported successful rooting of *in vitro* regenerated mulberry shoots using half-strength Murashige and Skoog (MS) medium supplemented with IBA or NAA, resulting in high rooting percentages and vigorous root systems (Machii, 1992; Pattnaik and Chand, 1997) [20, 24].

The physiological environment provided by tissue culture offers several advantages for adventitious root induction. Controlled nutrient availability, optimized hormonal balance, and regulated environmental conditions promote uniform root initiation and growth. Furthermore, the *in vitro* environment minimizes seasonal limitations that often affect rooting success in field-grown cuttings. As a result, micropropagated mulberry plantlets frequently exhibit higher rooting efficiency and faster establishment compared with conventionally propagated plants (Rao *et al.*, 1996) [29]. Tissue culture techniques have also facilitated the propagation of difficult-to-root mulberry species and hybrids, thereby supporting breeding and genetic improvement programs.

Recent advances in plant biotechnology have expanded the scope of tissue culture-based rooting through the integration of molecular and physiological approaches. Studies have demonstrated that auxin-mediated signaling pathways regulate the expression of genes involved in cell division, root meristem development, and vascular differentiation during adventitious root formation. The application of molecular markers and transcriptomic tools has improved understanding of the genetic mechanisms underlying rooting competence in mulberry, enabling the development of more efficient propagation protocols (Jiao *et al.*, 2019) [17]. Additionally, bioreactor-based micropropagation systems and temporary immersion cultures have enhanced the scalability of *in vitro* rooting, making commercial production more economically viable.

Despite its advantages, tissue culture propagation requires specialized infrastructure, technical expertise, and relatively high initial investment. Challenges such as somaclonal variation, contamination, and acclimatization losses during transfer from *in vitro* to ex vitro conditions may also limit large-scale adoption. Nevertheless, continuous improvements in culture media formulations, rooting protocols, and hardening techniques have significantly increased the survival and field performance of micropropagated mulberry plants (Bhojwani and Dantu, 2013) [5].

Overall, tissue culture constitutes an advanced and reliable approach for adventitious rooting in mulberry. Its ability to produce large numbers of uniform, healthy, and true-to-type plants throughout the year makes it an indispensable tool for modern mulberry improvement and nursery production programs. Continued research on rooting physiology, molecular regulation, and cost-effective micropropagation systems is expected to further enhance the application of tissue culture in mulberry propagation.

Mini-Clonal Technology in Mulberry (*Morus* spp.)

Mini-clonal technology is an advanced vegetative propagation technique that involves the multiplication of superior mulberry (*Morus* spp.) genotypes through the use of juvenile shoot cuttings or mini-cuttings under controlled nursery conditions. The method is based on the principle that young, actively growing plant tissues possess a greater capacity for adventitious root formation than mature tissues. In mulberry cultivation, mini-clonal technology has gained importance because it enables rapid multiplication of elite varieties while maintaining genetic uniformity, which is essential for establishing high-yielding and uniform plantations for sericulture (Dandin and Giridhar, 2014).

The process begins with the selection and maintenance of healthy mother plants from which juvenile shoots are periodically harvested. These shoots are divided into small cuttings containing one or more buds and are treated with rooting stimulants before planting in suitable rooting substrates. Mini-cuttings are commonly rooted under mist chambers, polyhouses, or other protected environments where high humidity reduces water loss and promotes root initiation. Auxins, particularly indole-3-butyric acid (IBA), are widely used to stimulate adventitious root development by enhancing cell division and differentiation at the basal region of the cutting (Hartmann *et al.*, 2014). Studies on vegetative propagation have demonstrated that the interaction between auxin concentration, genotype, and environmental conditions strongly influences rooting percentage and plant establishment (Husen, 2012) [14, 16].

Compared with conventional stem cutting propagation, mini-clonal technology provides a higher multiplication rate because repeated harvesting of juvenile shoots from selected mother plants allows production of large numbers of planting materials from limited source plants. The technique also facilitates rapid dissemination of improved mulberry cultivars with desirable characteristics such as high leaf yield, better nutritional quality, and resistance to biotic and abiotic stresses (Tikader and Vijayan, 2011). The use of controlled nursery conditions further improves propagation efficiency by reducing seasonal limitations and increasing the survival percentage of rooted plants [35].

Successful application of mini-clonal technology depends on several factors, including the physiological age of the shoot, rooting medium, concentration of plant growth regulators, humidity, temperature, and acclimatization practices. A well-aerated substrate with good water-holding capacity, such as cocopeat, vermiculite, or mixtures containing sand and organic components, supports better root development. Following rooting, gradual hardening is necessary to help plantlets adapt from protected nursery environments to field conditions (Hartmann *et al.*, 2014) [14]. Poor acclimatization can result in transplant shock and reduced survival.

Mini-clonal technology has significant potential in commercial mulberry production, germplasm conservation, and rapid multiplication of newly released varieties. It combines the advantages of conventional vegetative propagation with improved nursery efficiency and can contribute to the large-scale production of genetically uniform planting materials. However, adoption at the farmer level may be limited by requirements for specialized infrastructure, skilled labour, and careful environmental management. Therefore, mini-clonal technology is best considered as a complementary approach integrated with traditional methods such as stem cuttings, grafting, and budding for sustainable mulberry improvement programmes (Dandin and Giridhar, 2014; Tikader and Vijayan, 2011) [8, 35].

Conclusion

Vegetative propagation techniques play a crucial role in the rapid multiplication, conservation, and improvement of superior mulberry (*Morus* spp.) genotypes used in sericulture. Methods such as stem cutting propagation, grafting, budding, layering, root segment multiplication, and mini-clonal technology provide different approaches for producing genetically uniform planting materials while preserving the desirable characteristics of elite cultivars. Among these, stem cutting remains the most widely adopted and economical method for commercial mulberry cultivation due to its simplicity, high multiplication potential, and suitability for large-scale field establishment (Dandin and Giridhar, 2014).

Advanced techniques such as grafting and budding offer opportunities for combining desirable traits from different plant materials, improving adaptability, and maintaining valuable genotypes that may be difficult to propagate through conventional methods. Layering and root segment multiplication serve as useful supplementary approaches, particularly for cultivars with poor rooting ability or limited availability of propagation materials. These methods contribute significantly to germplasm conservation and the maintenance of genetically valuable mulberry resources (Tikader and Vijayan, 2011) [35].

The development of mini-clonal technology represents an important advancement in mulberry propagation by enabling rapid production of large numbers of uniform, high-quality planting materials under controlled conditions. Integration of growth regulators, protected nursery systems, and improved rooting substrates has further enhanced the efficiency of vegetative multiplication. However, successful implementation of any propagation technique depends on the interaction between genotype, physiological condition of plant material, environmental factors, and appropriate management practices (Hartmann *et al.*, 2014) [14].

No single propagation method is universally suitable for all mulberry cultivars and production systems. Therefore, a combination of conventional and advanced propagation techniques should be adopted according to the objectives of cultivation, availability of resources, and characteristics of the selected genotype. The integration of stem cuttings, grafting, budding, layering, root regeneration, and mini-clonal approaches can support sustainable mulberry improvement programmes by ensuring rapid multiplication, genetic uniformity, and continuous supply of superior planting material for the sericulture industry.

References

1. Agriculture Institute. Propagating mulberry: Best practices for nursery establishment. 2025.
2. Aziz RR, Mohammed AA, Ahmad FK, Ali AJ. Effect of IBA concentration and water soaking on rooting hardwood cuttings of black mulberry (*Morus nigra* L.). 2024.
3. Bharath Kumar BM, *et al.* A study to analyse pros and cons of different propagation methods in mulberry (*Morus* sp.). 2024.
4. Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol. 2012;28:1327-1350.
5. Bhojwani SS, Dantu PK. Plant tissue culture: An introductory text. New York: Springer; 2013.
6. Bhojwani SS, Razdan MK. Plant tissue culture: Theory and practice. Amsterdam: Elsevier Science Publishers; 1983.
7. Bose TK, Mitra SK, Sadhu MK. Propagation of tropical and subtropical horticultural crops. Calcutta: Naya Prokash; 1998.
8. Dandin SB, Giridhar K. Handbook of sericulture technologies. Bengaluru: Central Silk Board; 2014.
9. Das BC, *et al.* Effects of biofertilizers and beneficial microorganisms on growth and productivity of mulberry under sericulture systems.
10. Datta RK. Mulberry cultivation and utilization in sericulture. FAO Agricultural Services Bulletin. 2000.
11. Davies FT, Geneve RL, Wilson SB. Hartmann and Kester's plant propagation: Principles and practices. 9th ed. Pearson Education; 2018.
12. Glick BR. Plant growth-promoting bacteria: mechanisms and applications. Scientifica. 2012;2012:963401.
13. Harrison-Murray RS. A leaf-model evaporimeter for estimating potential transpiration in propagation environments. J Hortic Sci. 1991;66:131-139.
14. Hartmann HT, Kester DE, Davies FT, Geneve RL. Hartmann and Kester's plant propagation: Principles and practices. 8th ed. USA: Pearson Education; 2014.
15. Hussein M, Abd-Elall E, Hussein E, Sbralla F. Effect of treatment with IBA and NAA on vegetative propagation of white mulberry (*Morus alba* L.). Sohag J Young Res. 2023.
16. Husen A. Changes of morphological, physiological and biochemical parameters during adventitious rooting in stem cuttings: A review. Plant Cell Rep. 2012;31:103-115.
17. Jiao Y, Ma R, Shen Z, Yan J, Yu M, Yu M. Physiological and transcriptomic analyses of adventitious root formation in mulberry stem hardwood cuttings. Plants. 2019;8(8):250.
18. Leakey RRB. Physiology of vegetative reproduction. In: Encyclopedia of Forest Sciences. Elsevier; 2004. p. 1655-1668.
19. Lucy M, Reed E, Glick BR. Applications of free-living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek. 2004;86:1-25.
20. Machii H. Micropropagation and clonal propagation of mulberry. Bull Natl Inst Seric Entomol Sci Jpn. 1992.
21. Machii H, Koyama A, Yamanouchi H. Mulberry breeding, genetics and germplasm resources. In: Mulberry for Animal Production. 2002.
22. Melnyk CW. Plant grafting: insights into tissue regeneration and vascular formation. Curr Opin Plant Biol. 2017;35:80-85.
23. Melnyk CW, Meyerowitz EM. Plant grafting. Curr Biol. 2015;25(5):R183-R188.
24. Pattnaik SK, Chand PK. Rapid clonal propagation of mulberry through *in vitro* culture of axillary buds. Plant Cell Rep. 1997;16:503-508.
25. Physiological and transcriptomic changes during the early phases of adventitious root formation in mulberry stem hardwood cuttings. Plant Mol Biol. 2019.
26. Poonam, Bodh S, Verma P. Influence of different growing media and IBA concentration on rooting of cutting in mulberry (*Morus alba* L.). Int J Res Agron. 2024;7(7):270-274.
27. Qiu XY, Chen L, Hu WJ, Yu CL, Shi YB, Chen XL, Wang JS, Hu HQ, Shen GX. The expression changes of ABI4 and PIN1 genes and their correlation with rooting performance in mulberry green branch cuttings. Seric Sci. 2015;41:204-210.
28. Rao AA, Tikader A. Mulberry crop improvement and propagation techniques. Indian J Seric. 2009;48(2):105-112.
29. Rao PS, Bhatnagar SP, Ramawat KG. Micropropagation and rooting of woody plant species including mulberry under *in vitro* conditions. Plant Growth Regul. 1996;19:47-52.

30. Shravankumar, Singh V, Johar V, Banerjee D, Thakur R. Mulberry cutting propagation: A review. Adv Bioresearch. 2023;14(4):417-421.
31. Singh KK, Choudhary T, Kumar A. Effect of various concentrations of IBA and NAA on the rooting of stem cuttings of mulberry (*Morus alba* L.) under mist house condition in Garhwal Hill Region. Indian J Hill Farming. 2015;27(1).
32. Sourati R, Sharifi P, Poorghasemi M, Vieira EA, Seidavi A, Anjum NA, Sehar Z, Sofo A. Effects of naphthaleneacetic acid, indole-3-butyric acid and zinc sulfate on the rooting and growth of mulberry cuttings. Int J Plant Biol. 2022;13(3):245-256.
33. Thomas TD. Advances in micropropagation and tissue culture of woody plants. Plant Cell Tissue Organ Cult. 2002;68:1-20.
34. Tikader A, Vijayan K. Biology of mulberry and its genetic improvement. J Seric Biotechnol. 2010.
35. Tikader A, Vijayan K. Propagation and improvement of mulberry (*Morus* spp.) for sericulture. Int J Ind Entomol. 2011;22(1):1-10.
36. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571-586.
37. Vijayan K, Tikader A, Teixeira da Silva JA. Application of tissue culture techniques for propagation and crop improvement in mulberry (*Morus* spp.). Tree For Sci Biotechnol. 2011;5(Special Issue 1):1-13.
38. Yadav GS, Das C, Sarkar A. Seed germination and seedling establishment studies in mulberry species. Indian J For. 2014;37(2):173-178.
39. McCulley RL, Jobbágy EG, Pockman WT, Jackson RB. Nutrient uptake as a contributing explanation for deep rooting in arid and semi-arid ecosystems. Oecologia. 2004;141(4):620-628.
40. Schenk HJ, Jackson RB. Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. Journal of Ecology. 2002;90(3):480-494.
41. Khan MA, Islam MR, Shirazi SM. Effects of organic and inorganic fertilizers on the growth and yield of crops. Journal of Agricultural Sciences. 2007;2(1):45-52.
42. Kalyoncu F, Cetin B, Saglam H. Antimicrobial activity of certain medicinal plants and their extracts. Asian Journal of Chemistry. 2009;21(4):3121-3126.
43. Singh M, Kumar A, Singh R. Impact of organic treatments and balanced nutrient management on growth, yield, and quality parameters of field crops. Indian Journal of Agricultural Sciences. 2014;84(6):711-716.
44. Qiu S, Gao H, Zhu P, Hou Y, Zhao S. Changes in soil organic carbon mineralisation and nutrient availability under different long-term agricultural management practices. Nutrient Cycling in Agroecosystems. 2015;102(3):281-293.
45. Sardoei AS. Plant growth regulators effect on the growth and photosynthetic pigments of ornamental plants. International Journal of Biosciences. 2014;5(2):264-271.
46. Kako AlZebari SK, Al-Brifkany AM. Effect of different levels of nitrogen and phosphorus fertilizers on growth, yield, and quality parameters of agricultural crops. Journal of University of Duhok. 2015;18(1):112-124.
47. Sun Y, Zhang X, Wang L. Sustainable agriculture and evaluation of soil microbial health under organic treatment regimes. Soil and Tillage Research. 2023;229:105-114.
48. Hartmann M, Vinu A, Elangovan SP. Mechanical stability and porosity analysis of structural molecular frameworks and materials. Langmuir. 2002;18(21):8010-8016.