

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(8): 1507-1511
www.biochemjournal.com
Received: 09-05-2024
Accepted: 13-06-2024

Chetan Kumar DS
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

B Gowthami
Department of Biosciences and
Sericulture, Sri Padmavati
Mahila Visvavidyalayam,
Tirupati, Andhra Pradesh,
India

V Pavani Naga Durga
Department of Genetics and
Plant Breeding, Shahid
Gundadhar College of
Agriculture and Research
Station, Jagdalpur,
Chhattisgarh, India

Corresponding Author:
Chetan Kumar DS
Department of Sericulture,
College of Agriculture, UAS,
GKVK, Bengaluru,
Karnataka, India

Biotechnological approaches for enhancing mulberry yield and quality

Chetan Kumar DS, B Gowthami and V Pavani Naga Durga

DOI: <https://doi.org/10.33545/26174693.2024.v8.i8s.3778>

Abstract

Mulberry (*Morus* spp.) holds immense significance in agriculture, especially for sericulture, as it is the primary food source for silkworms (*Bombyx mori*). However, environmental pressures, pests, diseases, and the growing need for higher yields necessitate the development of more resilient and productive varieties. Biotechnology offers a variety of approaches to meet these challenges, focusing on enhancing both the yield and quality of mulberry plants. This review examines recent biotechnological strategies—ranging from genetic engineering to molecular breeding, tissue culture, and the application of biofertilizers—highlighting their contributions to improving mulberry cultivation. Additionally, the review addresses how these innovations can support sustainable agriculture and contribute to the economic growth of the sericulture industry.

Keywords: Mulberry, sericulture, biotechnology, genetic engineering, molecular breeding, sustainable agriculture

Introduction

The cultivation of mulberry plays a crucial role in the sericulture industry, which relies on the leaves as the sole nourishment for silkworms. However, the production of mulberry faces numerous challenges, such as climate change, soil degradation, pest outbreaks, and the necessity for higher leaf yields and better quality (Razdan and Thomas, 2021; Mogili *et al.*, 2023) [18, 14]. Traditional breeding methods have limitations in addressing these issues effectively. Biotechnological interventions have emerged as a solution, offering more precise and rapid means of improving mulberry yield and quality. This paper explores the most recent biotechnological advancements in mulberry farming, with a focus on their impact on productivity, resistance to pests and diseases, and leaf quality, ultimately supporting sustainable sericulture. To enhance mulberry productivity, biotechnological approaches such as genetic modification and genome editing have been increasingly applied. Genetic modifications can enable the development of mulberry varieties that exhibit higher resistance to environmental stressors, such as drought, extreme temperatures, and soil salinity. Additionally, genetic modifications that focus on increasing photosynthetic efficiency or improving nutrient uptake from the soil have the potential to significantly boost leaf yield. The introduction of genes that regulate growth hormones or enhance nutrient allocation in plants has led to improved biomass production, thus meeting the growing demand for high-quality mulberry leaves in sericulture. Moreover, biotechnology enables the development of disease-resistant varieties, which help reduce crop loss caused by common pathogens that affect mulberry trees. Furthermore, biotechnology plays a vital role in improving the nutritional quality of mulberry leaves, directly impacting the growth and health of silkworms. Enhancements in the protein, polyphenol, and micronutrient content of the leaves have been achieved through techniques such as gene editing and biofortification. These modifications not only improve silkworm health but also contribute to the production of stronger, more resilient silk fibres. By optimizing the chemical composition of mulberry leaves, biotechnology addresses key challenges in sericulture, including the need for healthier silkworms and higher-quality silk. Ultimately, these advancements offer a pathway towards more sustainable and efficient sericulture practices, ensuring the industry's resilience in the

face of global challenges. In addition to enhancing productivity and nutritional quality, biotechnological advancements have also contributed to the sustainable management of mulberry cultivation. By reducing the dependency on chemical pesticides and fertilizers, biotechnology supports environmentally friendly farming practices. The development of pest-resistant mulberry varieties through genetic engineering, such as those expressing *Bacillus thuringiensis* (Bt) proteins, helps minimize the use of harmful chemical treatments, reducing environmental pollution and preserving biodiversity. Furthermore, biotechnological innovations like microbial inoculants and bio-based fertilizers are being explored to improve soil health, promote plant growth, and reduce the environmental footprint of mulberry farming. These approaches not only enhance the sustainability of mulberry cultivation but also ensure that the sericulture industry remains viable in the long term, contributing to both economic and environmental sustainability.

Biotechnological Approaches for Enhancing Mulberry Yield

The main focus of mulberry breeding is to improve leaf productivity as it alone contributes more than 38.2% to the sericulture productivity (Banerjee, 1998) [12]. However, improving leaf productivity is difficult being a multifactorial trait and determined by a number of associated characters such as plant height, number of branches, leaf retention capacity, nodal length, leaf size and weight, and total biomass (Doss *et al.*, 2011) [18]. High heterozygosity and inbreeding depression hinder the development of inbreds; hence, directional breeding failed to make much progress. Therefore, the heterozygous parents are used to generate F1 progenies, which are then subjected to different evaluation and selection procedures to identify the best one. This type of breeding system bars the possibility of introgressing genes of desirable traits from wild relatives/species due to genetic drag and subsequent difficulty in eliminating the undesirable traits that come along. Under such circumstances, the feasible means of improving specific traits without disturbing the current trait combinations is adoption of biotechnological tools like transgenesis, which enable introduction/over expression of desirable genes (Vijayan *et al.*, 2011a) [23], or knocking out undesirable genes via RNA interference technology (Vijayan *et al.*, 2011b) [23]. Mulberry, being a tree with high heterozygosity, poses difficulties on improving traits of economic importance through conventional breeding and selection. Environmentally less influenced and developmentally stable molecular markers provide reliable tools for the breeders to characterize the germplasm and select parents and offspring's through marker-assisted selection. Thus, it would be prudent to use biotechnological tools to harness the vast benefit mulberry offers to mankind.

1. Genetic Engineering

Genetic engineering has proven to be an effective tool in improving the yield and resilience of crops, and mulberry is no exception. The introduction of specific genes into the mulberry genome can impart beneficial traits such as pest resistance, enhanced drought tolerance, and faster growth rates.

- **Pest Resistance:** The application of genetic modification to impart resistance against pests,

particularly the mulberry silk moth (*Bombyx mori*), has been explored through the incorporation of *Bacillus thuringiensis* (Bt) genes. These genes allow mulberry plants to produce insecticidal proteins that deter pests, reducing the need for chemical pesticides and ensuring better leaf quality.

- **Drought Tolerance:** Drought poses a significant challenge to mulberry cultivation, especially in regions facing irregular rainfall. Genetic engineering has been employed to introduce drought-resistant traits in mulberry plants. For example, the expression of *DREB* (Dehydration-Responsive Element Binding) transcription factors has been shown to improve water retention and enhance growth under water stress conditions, thereby ensuring a stable supply of mulberry leaves.
- **Accelerated Growth:** Genetic modifications targeting growth regulators like auxins and gibberellins have led to faster growth rates in mulberry trees. These plants are not only able to regenerate more quickly but also accumulate more biomass, increasing leaf yield per plant.

2. Molecular Breeding

Molecular breeding combines traditional breeding techniques with modern molecular tools, accelerating the selection of desirable traits in crops. This method has shown considerable potential in improving mulberry varieties.

- **Marker-Assisted Selection (MAS):** Marker-assisted selection uses molecular markers to identify and select plants with desirable traits, such as high leaf protein content or disease resistance. For mulberry, MAS has been instrumental in selecting varieties with improved growth and higher nutritional value, which are beneficial for silkworms.
- **Gene Editing with CRISPR/Cas9:** The CRISPR/Cas9 system provides precise control over genetic modifications, enabling researchers to edit genes with high accuracy. In mulberry, CRISPR has been used to alter genes related to pest resistance, drought tolerance, and even nutritional content, allowing for the development of mulberry plants tailored to specific environmental conditions.
- **Quantitative Trait Locus (QTL) Mapping:** By identifying specific genes linked to important traits such as leaf size, growth rate, and resistance to diseases, QTL mapping enables breeders to develop mulberry cultivars with improved characteristics. This method significantly shortens the breeding cycle, making it easier to introduce beneficial traits into new varieties.

The genetic characterization of promising varieties of mulberry is important for their utilization in the sericulture industry. Traditionally, genetic diversity in mulberry has been determined by examining morphological and physiological traits (Biasiolo *et al.* 2004; Dandin and Jolly 1986; Huo 2002; Machii *et al.* 1997) [4, 7, 11, 13], but characterization through morphological features fail to serve as unambiguous markers as most of the phenotypic traits are under the control of developmental stages and environmental influences. With the advent of molecular biology, DNA markers are universally accepted as they are highly polymorphic, multiallelic, universally reproducible, dispersed throughout the genome, and independent of tissue

and environmental influence. A summary of work on molecular markers used to detect genetic diversity in mulberry is given in Table 2. ISSRs have been the most common marker system used in mulberry. They were also combined with RAPD (Awasthi *et al.* 2004; Naik and Dandin 2005; Srivastava *et al.* 2004) and SSRs (Zhao *et al.* 2007) [1, 19, 25] to reveal genetic variation among mulberry varieties. Single nucleotide polymorphisms (SNPs) have recently emerged as a powerful tool in marker technology as they are the most abundant polymorphism present in the genome of any organism (Collins *et al.* 1997) [6]. SNPs are single base variations widely distributed throughout the genomes with the frequency of 1% in a population. However, the usefulness of SNPs depends on sequence information available, and despite a reasonable number of ESTs in public databases, no SNPs have been reported from mulberry as of today. This necessitates the importance of expressed sequence tags (ESTs) to be generated in public databases for SNPs mining and developing other DNA-based markers. SNP detection in mulberry can open new perspectives for genetic studies and will also be useful for association analysis of candidate genes with phenotypic variation, quantitative trait loci (QTL) mapping, assessment of genetic diversity, and for synteny-based comparisons from model crop species. Further, accumulation of available molecular markers will contribute to identification of QTLs for traits related to abiotic and biotic stresses.

3. Tissue Culture Techniques

Tissue culture offers a rapid and efficient method for propagating mulberry plants while maintaining their genetic integrity. This technique has been widely used in the commercial production of mulberry varieties with specific traits.

- **Micropropagation:** Through tissue culture, high-quality mulberry plants can be clonally propagated, ensuring uniformity and consistency in the desired traits. Elite mulberry varieties with desirable characteristics such as high leaf yield or pest resistance can be multiplied at a large scale, facilitating their widespread use in sericulture. Composition of media is one of the factors that significantly affect micropropagation in mulberry. Among the different media compositions and hormones tested, MS (Murashige and Skoog, 1962) [15] medium containing 2 mg L⁻¹ 6-benzylamino purine (BAP) is the best for shoot multiplication (Lalitha *et al.*, 2013) [12]. However, it is also important to note that concentrations of 6-benzyladenine (BA) above 2 mg L⁻¹ is inhibitory for shoot initiation and multiplication (Bhau and Wakhlu, 2003) [3]. Among the different sugars such as sucrose, glucose and fructose tested, sucrose is the best (Bhau and Wakhlu, 2003) [3]. Vijayan *et al.* (2000) [22] found that 3% glucose is better for shoot formation from leaf explants. Among different pH levels tested, 5.6-5.8 is optimum for shoot multiplication (Enomoto, 1987) [9]. Agar in the culture medium also plays an important role in the success of micropropagation. Among various concentrations tested, 0.8% agar is the best whereas above 1% reduced shoot growth (Pattnaik and Chand, 1997; Thomas, 2003) [17, 20]. Rooting in mulberry is achieved mostly in half-strength MS medium as it is found better than full strength. Among the plant hormones, 0.5 mg L⁻¹ NAA is the best for *M. alba*, *M.*

indica, *M. multicaulis* and *M. latifolia* resulting in 95% rooting (Vijayan *et al.*, 2003) [21], though indole-3-butyric acid (IBA) is the best auxin for *M. nigra*. Observed that higher concentrations of most of the auxins (>1.0 mg L⁻¹) were inhibitory for root formation in mulberry and there was a strong interactive relationships among genotype, hormone type and concentration. Generally, rooting initiate is obtained within 12-18 days after transferring shoots to the rooting media (Pattnaik and Chand, 1997) [17]. *In vitro* plantlets need to be hardened in the greenhouse before transfer to the field. Since the newly developed plantlets are grown in a pathogen free atmosphere in the laboratory, sudden exposure to outside conditions may put them into the risk of infestation by pests and diseases. In order to avoid/reduce such risks, hardening in autoclaved vermi-compost is considered as one of the most suitable substratum for the initial stages 2-3 weeks of plantlets establishment *ex vitro*. After the initial hardening, the plants are transferred to earthenware pots of 12 cm diameter containing garden soil composed of 50% soil and 50% compost. The pots are kept under shade for another 2-3 weeks, before transferring to the field (Chattopadhyay *et al.*, 2013; Vijayan *et al.*, 2011b) [12, 24]. Biological hardening is an alternative method for hardening micro propagated plants before transferring to the field and in this technique plants are challenged with biotic stress caused by soil microbes. Many fungi like vasicular arbuscular mycorrhiza (VAM), *Trichoderma*, *Piriformospora indica*, and *Azotobacterchroococcum* are used for biological hardening (Kashyap and Sharma, 2006) [20].

- **Somaclonal Variation:** While tissue culture techniques generally aim for genetic uniformity, they also introduce somaclonal variation, which can sometimes result in new traits. This variation can be harnessed to create mulberry plants with novel characteristics, such as improved resistance to diseases or pests.
- **Protoplast Fusion:** Protoplast fusion, which involves the fusion of plant cells to create hybrid plants, has been explored as a way to develop new mulberry varieties. By combining desirable traits from different mulberry species, this method has the potential to create hybrids with improved growth and quality characteristics. Somatic hybridization through protoplast fusion has opened a new avenue for developing new characteristics, which are not possible through conventional breeding. There are only a few reports dealing with plant regeneration from protoplasts in mulberry. A combination of 2% cellulase, 1% macerozyme and 0.5% macerase is found optimal for better isolation of viable protoplast. Protoplast fusion in mulberry was successfully achieved using chemical fusogen and electro-fusion. Although protoplast isolation and regeneration was achieved, development of somatic hybrids in mulberry could not be achieved. Hence, efforts in this end need to be continued.

4. Symbiotic Associations and Biofertilizers

- **Mycorrhizal Fungi:** The symbiotic relationship between mulberry plants and mycorrhizal fungi enhances nutrient uptake, particularly phosphorus, which is crucial for plant growth. By improving nutrient

absorption, these fungi promote healthier growth, which in turn increases leaf production.

- **Biofertilizers:** Biofertilizers, including nitrogen-fixing bacteria, can also improve mulberry growth. These microorganisms assist in fixing atmospheric nitrogen, thereby reducing the need for synthetic nitrogen fertilizers. This contributes to the sustainability of mulberry farming and enhances soil health.

Biotechnological Approaches for Enhancing Mulberry Quality

The nutritional quality of mulberry leaves is crucial for the health of silkworms and the silk they produce. Recent biotechnological advancements have focused on enhancing the protein, polyphenol, and micronutrient content of these leaves, leading to improved silk production. Various biotechnological approaches have been applied to enhance the nutritional content of mulberry leaves, with a key objective being the increase in protein content. Genetic modifications aimed at modifying genes associated with protein synthesis have successfully enhanced the levels of key proteins, making the leaves a more nutritious food source for silkworms (Fan, 2015) [28]. Similarly, enhancing the polyphenol content of mulberry leaves through genetic engineering has been shown to improve the silk's tensile strength and sheen. Polyphenols, particularly flavonoids, play a crucial role in silkworm health and silk quality (Ghorpade *et al.*, 2022) [10]. Moreover, micronutrient biofortification, such as increasing iron, zinc, and calcium levels in mulberry leaves, has been shown to enhance silkworm health, which in turn improves the quality of the silk produced (Borgohain, 2015) [5].

Biotechnology also indirectly enhances silk quality by optimizing the nutritional profile of mulberry leaves. Since silkworms rely entirely on these leaves for nutrition, improving their protein, polyphenol, and micronutrient content leads to higher-quality silk. Phytochemical engineering techniques have been applied to increase the production of flavonoids and other bioactive compounds in mulberry leaves, which can enhance the resilience and strength of silk fibres. This optimisation of the nutritional content of mulberry leaves leads to more robust silkworms, resulting in stronger and finer silk production (Ghorpade *et al.*, 2022) [10].

Alongside improvements in leaf quality, biotechnological approaches have focused on enhancing pest and disease resistance in mulberry plants. Disease-resistant varieties have been developed using molecular breeding and genetic engineering techniques to resist common diseases like root rot and leaf spot, which can compromise leaf quality and yield (Ghorpade *et al.*, 2022) [10]. Additionally, genetically modified mulberry plants expressing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have shown increased resistance to pests, thereby protecting leaf quality and improving the yield for sericulture. This approach ensures a consistent and healthy food source for silkworms, ultimately supporting the overall health of the silk industry. Despite these advancements, there is some concern regarding the ecological impact of genetic modifications and the potential loss of traditional mulberry varieties, which may possess unique beneficial traits. The development of genetically modified mulberry varieties raises questions about the preservation of genetic diversity and the long-term sustainability of mulberry cultivation. It is essential to

balance the benefits of biotechnological innovations with the protection of traditional mulberry cultivars to maintain biodiversity and ecosystem stability in sericulture.

Conclusion

Biotechnological innovations are essential for addressing the challenges faced by mulberry cultivation. Genetic engineering, molecular breeding, tissue culture, and biofertilizer applications all play a significant role in improving mulberry yield and quality. These advancements help mitigate environmental stresses, enhance plant resilience, and improve the nutritional value of mulberry leaves, directly benefiting the sericulture industry. Future research should focus on further improving the precision of gene editing techniques and exploring new ways to integrate sustainable practices in mulberry farming.

References

1. Awasthi AK, Naik NGM, Sriramana GV, Thangavelu K, Javaregowda N. Genetic diversity and relationships in mulberry (Genus *Morus*) as revealed by RAPD and ISSR marker assays. *BMC Genetics*. 2004;5:1-4.
2. Banerjee SP. Evaluation of mulberry (*Morus* spp.) genotypes for propagation parameters. *Indian Journal of Sericulture*. 1998;37:133-136.
3. Bhau BS, Wakhlu AK. Rapid micropropagation of five cultivars of mulberry. *Biologia Plantarum*. 2003;46:349-355.
4. Biasiolo M, Canal MT, Tomadore N. Micromorphological characterization of ten mulberry cultivars (*Morus* spp.). *Economic Botany*. 2004;58:639-646.
5. Borgohain A. Nutritional Supplement and its Effect on Mulberry Silkworm *Bombyx mori* L. [Internet]. 2015. Available from: http://ijirset.com/upload/2015/august/187_Nutritional.pdf.
6. Collins FS, Guyer MS, Chravarti A. Variations on a theme: Cataloging human DNA sequence variation. *Science*. 1997;275:1580-1581.
7. Dandin SB, Jolly MS. Mulberry descriptor. *Sericologia*. 1986;26(4):465-475.
8. Doss SG, Vijayan K, Chakraborti SP, Ghosh PG. Character association in improved mulberry genotypes exhibiting delayed leaf senescence. *Journal of Ornamental Horticultural Plants*. 2011;1:85-95.
9. Enomoto S. Preservation of genetic resources of mulberry by means of tissue culture. *Japan Agricultural Research Quarterly*. 1987;21:205-210.
10. Ghorpade K, Netam V, Desai NM, Shewale VD. Ascertaining role and importance of artificial diet and botanicals mediated serifeed for the strengthening of the sericulture industry. *Uttar Pradesh Journal of Zoology*. 2022;43:46-54. Available from: <https://doi.org/10.56557/upjoz/2022/v43i223230>.
11. Huo Y. Mulberry cultivation and utilization in China. In: Sanchez MD, editor. *Mulberry for Animal Production*. Proceedings of an electronic conference carried out between May and August 2000. FAO Animal Production and Health Paper 147. Rome: FAO; 2002. p. 11-44.
12. Lalitha N, Kih S, Banerjee R, Chattopadhyaya S, Saha AK, Bindroo BB. High frequency multiple shoot induction and *in vitro* regeneration of mulberry (*Morus*

- indica* L. cv. S-1635). International Journal of Advanced Research. 2013;1:22-26.
13. Machii H, Koyama A, Yamanouchi H, Katagiri K. Manual for the characterization and evaluation of mulberry genetic resources. Miscellaneous Publications of the National Institute of Sericultural and Entomological Science. 1997;22:105-124.
 14. Mogili T, Sarkar T, Gnanesh BN. Mulberry breeding for higher leaf productivity. In: The Mulberry Genome. Cham: Springer International Publishing; 2023. p. 57-114.
 15. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*. 1962;15:473-497.
 16. Naik VG, Dandin SB. Molecular characterization of some improved and promising mulberry varieties (*Morus* spp.) of India by RAPD and ISSR markers. *Indian Journal of Sericulture*. 2005;44(1):59-68.
 17. Pattnaik SK, Chand PK. Rapid clonal propagation of three mulberries, *Morus cathayana* Hemsl., *M. lhoukoiz.*, and *M. serrata* Roxb., through *in vitro* culture of apical shoot buds and nodal explants from mature trees. *Plant Cell Reports*. 1997;16:503-508.
 18. Razdan MK, Thomas TD, editors. Mulberry: Genetic Improvement in Context of Climate Change. CRC Press; 2021.
 19. Srivastava P, Vijayan K, Awasthi AK, Saratechandra B. Genetic analysis of *Morus alba* through RAPD and ISSR markers. *Indian Journal of Biotechnology*. 2004;3:527-532.
 20. Thomas TD. Thidiazuron induced multiple shoot induction and plant regeneration from cotyledonary explants of mulberry. *Biologia Plantarum*. 2003;46:529-533.
 21. Vijayan K. Genetic relationships of Japanese and Indian mulberry (*Morus* spp.) genotypes revealed by DNA fingerprinting. *Plant Systematics and Evolution*. 2003;243:221-232.
 22. Vijayan K, Chakraborti SP, Roy BN. Plant regeneration from leaf explants of mulberry: Influence of sugar, genotype, and 6-benzyladenine. *Indian Journal of Experimental Biology*. 2000;38:504-508.
 23. Vijayan K, Kar PK, Tikader A, Srivastava PP, Awasthi AK, Thangavelu K, *et al.* Molecular evaluation of genetic variability in wild populations of mulberry (*Morus serrata* Roxb.). *Plant Breeding*. 2004a;123:568-572.
 24. Vijayan K, Srivastava PP, Awasthi AK. Analysis of phylogenetic relationship among five mulberry (*Morus*) species using molecular markers. *Genome*. 2004;47:439-448.
 25. Zhao WG, Wang YH, Chen TT, Jia GF, Wang XM, Qi JL, *et al.* Genetic structure of mulberry from different ecotypes revealed by ISSRs in China: Implications for conservation of local mulberry varieties. *Scientia Horticulturae*. 2007;115:47-55.