

## Biotechnological Advancements in Sericulture

Guruprasad H.

Assistant professor (Entomology), UAS, Raichur

Corresponding Author: [hiremath1975@gmail.com](mailto:hiremath1975@gmail.com)

### Introduction

Sericulture, the science of silk production, provides employment to around 6 million people in India, contributing significantly to the economy. Since independence, India's silk production has grown from 11,000 MT in 1989–1990 to 31,906 MT in 2017–2018, making it the second-largest producer globally (4). The major thrust areas for sericulture are the production of superior mulberry and silkworm varieties for the tropical conditions of the country, identification of new silkworm rearing technologies, suitable methods for containment of pests and diseases in sericulture etc.(3). Given the impact of climate change and farming complexities, there is a growing need for advanced scientific approaches. Biotechnology has emerged as a powerful tool to enhance sericulture productivity and sustainability. Silk, a high-value natural fiber, is in increasing demand, driven by its use in textiles, biomaterials, and as a platform for foreign protein production through silkworms.

Recent innovations in biotechnology, such as molecular markers, transgenic technologies, and artificial feed development, have contributed to improvements in mulberry and silkworm varieties, disease management, and silk-based biomaterials. Additionally, *Morus alba*, the primary silkworm food source, has shown potential in pharmacological applications, such as antidiabetic and anticancer properties. By integrating modern biotechnological approaches, including genetic improvements, biomaterials, and pest control, sericulture can meet the growing demand for silk and overcome existing challenges. This review highlights the potential of biotechnology in revolutionizing the sericulture industry.

### Molecular markers for mulberry improvement

The principal application of mulberry plants in sericulture is the use of their leaves to rear silkworms for silk production. However, mulberry cultivation represents over 60% of the total cost in cocoon production, underscoring the need for developing high-yielding mulberry varieties. Traditional breeding strategies have resulted in several high-

yielding varieties, but challenges such as limited genetic information, lack of pure lines, dioecious nature, long juvenile periods, and absence of efficient screening methods have hindered progress. To overcome these, modern biotechnological tools, particularly molecular markers, are being applied in mulberry improvement. Molecular markers such as single nucleotide polymorphism (SNP), Inter simple sequence repeats (ISSR), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) facilitate faster and more precise identification of genotypes, characterization of germplasm, and analysis of genetic diversity. Marker-assisted selection (MAS) offers advantages over conventional breeding, enabling early selection, reducing the need for large populations and multiple generations, and enhancing efficiency.

Molecular markers have been extensively used for genetic diversity studies, germplasm characterization, and trait mapping in mulberry. The development of QTL maps for traits such as water use efficiency (WUE), root traits, and yield has been explored, though a direct link between specific markers and agronomic traits is still developing. The whole-genome sequencing of *Morus notabilis* has further advanced mulberry breeding, providing valuable genomic resources for future research.

### Tissue culture in mulberry genetic improvement

Tissue culture is a key tool for genetic improvement in mulberry, addressing challenges such as its perennial nature, long juvenile period, and limited propagation methods. Conventional propagation through stem cuttings or grafting is slow and dependent on various factors like plant age, genetics, and climate. Tissue culture, offering the advantage of clonal propagation, allows for faster multiplication and the development of desirable traits. Techniques like micro propagation, callus culture, organogenesis, and somatic embryogenesis have been widely used to improve mulberry varieties, including the production of haploids, triploids, and stress-

tolerant genotypes. Tissue culture also facilitates the enhancement of secondary metabolites and the application of transgenic methods, advancing both mulberry breeding and sericulture productivity.

### **Molecular markers in silkworm improvement**

Molecular markers have significantly advanced silkworm genetic improvement in India, particularly for enhancing traits like thermo tolerance, disease resistance, and cocoon yield. Traditionally, silkworm breeding relied on morphological traits, which are environmentally influenced, but molecular techniques like Marker-Assisted Selection (MAS) provide a more accurate and reliable approach. Research has led to the identification of over 400 mutations in *Bombyx mori* and the development of linkage maps using DNA markers such as RFLPs, RAPD, ISSR, and microsatellites. The creation of the *SilkBase* genome database and the use of Expressed Sequence Tags (ESTs) have further aided in mapping important traits, including sex-linked markers and those related to cocoon yield. Studies on tasar silkworms (*Antheraea mylitta*) have also used molecular markers to understand genetic diversity and population structure. Notably, the Central Silk Board in India has made strides in developing thermotolerant silkworms for tropical climates, with microsatellite markers linked to this trait.

### **Transgenic research in sericulture**

Transgenic research in sericulture utilizes modern biotechnology and genetic engineering to introduce desirable traits into mulberry and silkworms. By using recombinant DNA technology, scientists can isolate, modify, and insert genes from unrelated organisms to overcome the limitations of conventional breeding. This has led to significant advances, such as the development of transgenic silkworms producing spider silk, which has revolutionized the silk industry. Transgenic approaches offer a powerful tool for enhancing silk quality and other traits in sericulture, contributing to improved production and new applications.

**Transgenic mulberry:** Transgenic mulberry research focuses on improving stress tolerance, particularly against drought, salinity, and fungal pathogens. Although transformation protocols using particle bombardment and *Agrobacterium tumefaciens* have been developed for *Morus indica*, challenges remain in optimizing these methods. Successful transgenic

mulberry varieties have been developed with genes such as osmotin, which enhances tolerance to abiotic stresses like drought, and the *Hva1* gene from barley, which confers resistance to drought, salinity, and cold. These advancements offer potential for improving mulberry productivity under challenging environmental conditions.

### **Transgenic silkworm**

Transgenic silkworm research has made significant strides, particularly through the use of the Piggy Bac DNA transposon for germ line transformation of *Bombyx mori*. This method enables the insertion of target genes into the silkworm genome, allowing for the production of recombinant proteins and high-quality silk. Recent developments include the use of transgenic silkworms as bioreactors to produce monoclonal antibodies, cancer drugs, and glycoproteins with reduced antigenicity. Additionally, transgenic silkworms are being explored for the production of recombinant human serum albumin (HSA), opening new possibilities for both sericulture and biotechnology.

### **Artificial diet in sericulture**

Artificial diets have become a key solution in sericulture, addressing challenges such as variability in leaf quality, weather-related issues like wet leaves during rainy seasons, shrinking farmland, and labor shortages. Early attempts at rearing silkworms on artificial diets began in the 1960s, and since then, various formulations have been developed, such as pellet diets and Yuner diets, which are designed to be fed to silkworms after soaking or mixing with hot water. Research has shown that these diets can match or even surpass mulberry leaf feeding in terms of cocoon quality and yield. For instance, the semi-synthetic diet *Seri Nutrid* has yielded better cocoon weights and shell ratios compared to mulberry leaves. Additionally, probiotics and nutritional supplements have been explored to further enhance silkworm growth and productivity. In the case of non-mulberry silkworms like *Antheraea mylitta* (tasar silkworm), artificial feeds such as *Tasar Amrit* have been developed for indoor rearing, showing improved survival rates and rearing effectiveness compared to outdoor rearing on natural food sources. These innovations in artificial feeding have opened new avenues for sericulture, particularly in improving silkworm productivity and sustainability.

## Application of biotechnology in silkworm disease management

Silkworm diseases, such as grasserie, flacherie, muscardine, and pebrine, caused by viruses, bacteria, fungi, and microsporidia, have long been a significant issue in sericulture, leading to substantial cocoon losses. Traditional methods for disease management include preventive measures and diagnostic techniques like ELISA, DNA hybridization, and monoclonal antibody-based assays. However, modern biotechnology is playing an increasingly vital role in improving disease detection and control. Rapid diagnostic tools, particularly PCR-based methods, have been developed for early detection of pathogens such as viruses and microsporidia. Emerging techniques like Loop-Mediated Isothermal Amplification (LAMP) for pebrine detection and RNA interference (RNAi) for engineering viral resistance are opening new avenues for more effective silkworm disease management. These advancements help reduce crop loss and enhance sericulture productivity.

## Silkworm as bioreactor for expression of foreign protein

Silkworms, particularly *Bombyx mori*, are increasingly being used as bioreactors for the large-scale production of pharmaceutically important proteins and bio molecules. Genetic engineering advancements have enabled the expression of foreign proteins in silkworm larvae or cocoons, offering a cost-effective alternative to cultured cell lines. Early successes, such as the production of human  $\alpha$ -interferon using the *BmNPV* (*Bombyx mori* nucleopolyhedrovirus) expression system, have paved the way for producing other proteins like recombinant erythropoietin (rEpo) and bovine interferon- $\tau$ . The development of the Bac-to-Bac system, which allows the replication of recombinant baculoviruses in *E. coli*, has streamlined the process, overcoming previous limitations. Silkworms have been used to produce vaccines, like foot-and-mouth disease virus (FMDV) capsid proteins, and other valuable bio molecules. This approach holds significant potential for India, leveraging its rich silkworm-rearing heritage for biotechnological applications.

## Silk based biomaterials

Silk-based biomaterials, derived from the four types of silk produced in India, have gained significant attention for non-textile applications,

particularly in medical and cosmetic fields. Silk proteins, such as sericin and fibroin, are used in creating hydrogels, scaffolding matrices, films, and nanofibrous mats, showing promise in wound healing, skin care, and tissue engineering. Sericin, for example, has moisturizing properties and supports skin hydration, while fibroin is used in wound healing applications, such as films and sponges, due to its collagen-stimulating effects. Silk fibroin has also been explored in the creation of corneal films, cardiac patches, and artificial blood vessels. Additionally, silk proteins are utilized in orthopaedic and dental applications, such as osteoblast adhesion and as carriers for antibiotics like gentamycin. These developments indicate the potential of silk-based biomaterials in diverse medical applications, offering new opportunities for the sericulture industry.

## Waste Material in Sericulture

The silk industry generates several by-products, with silkworm pupae being one of the most significant. These pupae, produced after the silk threads are extracted from the cocoons, are typically discarded, although they are rich in essential nutrients, including proteins, lipids, and minerals. Each year, approximately 40,000 metric tons of silkworm pupae are produced in India alone, which are often wasted through drying and disposal methods that contribute to environmental pollution. Despite this, silkworm pupae have significant untapped potential. They contain 55-60% protein, which is highly nutritious and can be used as a protein source for human consumption. They also have high antioxidant properties, which can be utilized in cosmetic formulations. The oil extracted from pupae is enriched with  $\alpha$ -linolenic acid, making it valuable for oleochemical and food processing industries. Beyond human consumption, silkworm pupae can be used in animal feed, particularly for poultry and fish farming. Additionally, the pupae can serve as a base material for the production of mushrooms. Other components like chitin and chitosan, found in the skin of the pupae, are valuable for their antimicrobial properties and biodegradability. Overall, silkworm pupae represent a valuable resource that can contribute to sustainable practices in the silk industry while also providing significant nutritional and economic benefits.

**Conclusion:** Sericulture is evolving beyond silk production for textiles into more sustainable and

profitable avenues, including non-textile applications. Biotechnology is key to this transformation, driving innovations in genetic improvements, disease resistance, biomolecule synthesis, and waste utilization. Advances in silk-based biomaterials and biomedical applications offer new commercial opportunities. By integrating biotechnology, sericulture can enhance productivity, sustainability, and ecological balance, paving the way for future growth and diversification in the silk industry.

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