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A Review on Recent Advances in Biotechnology for Sustainable Sericulture

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Introduction:

Sericulture is one of the oldest agro-based industries in the world and has played a pivotal role in the economic development of several countries for more than four thousand years. The industry encompasses the cultivation of mulberry (*Morus* spp.), rearing of the domesticated silkworm (*Bombyx mori*), cocoon production, silk reeling, weaving, and value addition. Countries such as China and India dominate global silk production, collectively contributing more than 90% of the world's raw silk. Beyond its economic significance, sericulture provides year-round employment opportunities, particularly for women and smallholder farmers, making it an important component of rural livelihood security and poverty alleviation (Sarkar *et al*., 2000). The increasing global demand for premium-quality natural silk, coupled with concerns regarding environmental sustainability, has prompted researchers to develop innovative technologies for improving productivity and quality. Conventional sericulture practices rely heavily on selective breeding, manual disease diagnosis, traditional mulberry cultivation, and labor-intensive management systems. Although these approaches have contributed significantly to the industry's growth, they are often constrained by slow genetic improvement, susceptibility to diseases, climate-induced stress, declining soil fertility, and inconsistent cocoon quality. Frequent outbreaks of viral, bacterial, fungal, and protozoan diseases continue to cause substantial economic losses in silkworm production across many sericulture regions (Agarwal *et al*., 2004). Silk fibroin shows remarkable mechanical, degradation and biocompatibility properties, favoring its use to generate highly loaded grafts, especially in the musculoskeletal field. The sericin is a natural polymer, which acts as an adhesive joining two fibroin filaments in order to form silk yarn. Sericin has unique properties such as an antioxidant, moisturizing, healing, antibacterial, antimicrobial protection against ultraviolet radiation and anti-tumor. The advent of genetic engineering techniques such as transgenic technology, bioengineering, nanotechnology etc. has

enabled us to transform the silkworm's genetic makeup in a variety of novel ways. The silkworm, *Bombyx mori* L. has become a useful model of the Lepidoptera, with an increasingly important role in basic biological research. Silk produced from silkworm cocoons exist in fibre form in nature but the artificial engineering techniques facilitate the fabrication of silk solutions from silk fibre inversely. These silk nanostructures can be utilized to fabricate structurally and functionally efficient materials such as hydrogels, fibres, films, coatings and 3D matrices. Silk materials possess manifold applications in biomedicine and emerging fields such as water treatment, environmentally adaptive materials, and optical and electronic devices. After the invention of the *piggyBac* transposon mediated technology to make silkworm transgenic, efforts have been made to study the silk gland of the silkworm and to make it a suitable efficient system for the production of the foreign protein. In past decade more than 10 foreign proteins were successfully expressed in transgenic silkworm which includes model protein, human or animal derived pharmaceutical proteins and silk- based proteins. In several recent scientific talks, the applicability and potential of biotechnology in boosting sericultural output have been unambiguously accepted. It has been shown to be an effective instrument for the silk industry's and sericulture's long-term growth. One of the most valuable natural fibers is silk, which costs around 20% more per unit than raw cotton. The use of modern biotechnological tool for improvement of silkworm, host plant, development of artificial feed, seri waste utilization, production of silk-protein based biomaterials etc. holds great potential not only to meet production demand of silk but also to improve the industry in a com prehensive manner.

Biotechnology Advances and Silkworm Improvement:

Targeted modification of silkworm genomes has been made possible by biotechnology tools like transcriptomics, functional genomics, marker-assisted breeding, quantitative trait loci mapping, genomic selection, and genetic engineering (transgenesis and gene editing) to instill desired quantitative and qualitative traits of importance beyond the conventional characteristics. To create superior silkworm strains, researchers have successfully inserted genes to improve the strength, color, and pathogen resistance of silk fibers. Cocoon crop improvement is being accelerated by the use of molecular biology techniques in conjunction with conventional genetic procedures, which enable breeders to make well-informed decisions. Genome sequencing technology has led to fast advancements in sericulture, and genetic and genomic techniques have broader applicability. DNA sequences, biochemical traits (such as protein

structure or isoenzyme properties), physiological traits (such as growth rate or resistance to abiotic stress), and morphological traits are examples of how genetic differences between individuals are expressed (Rao and Hodgkin, 2002). From the perspective of artificial selection and evolution, the distribution and variability of microsatellite sequences within a species' genome might provide insight into its genetic past. For the analysis of pedigree, population structure, genome variation, evolutionary processes, and fingerprinting purposes, microsatellites are an appropriate genetic marker (Muneer, 2014). Microsatellite loci are longer and more variable in the species from which they are derived (the focal species) than similar loci in other (non-focal) species, according to numerous interspecific comparisons.

Tissue Culture in Mulberry Genetic Improvement:

In addition to enhancing food security and promoting socioeconomic development, the primary uses of plant biotechnology are to support the protection, diversification, and sustainable use of plant genetic resources for food and agriculture. The purpose of plant biotechnology and its use of cutting-edge techniques in sericulture is to enhance productive traits and their ability to withstand stress. Furthermore, the plant exhibits significant heterozygosity, and cross-pollination is the norm rather than the exception. The species has 28 diploid (2n) chromosomes, however it can also have extremely polyploid numbers up to 308. Among the polyploids, the triploids have a number of advantageous characteristics, such as quality and disease and cold resistance. However, triploids require a lot of time to produce and multiply. An alternative to the conventional vegetative propagation of woody species with such desirable features is the growing use of tissue culture technologies in clonal propagation. Actually, there are unmatched prospects for improving trees through the in vitro development of cells, tissues, and organs. The idea of totipotency, first put forth by Haberlandt in 1902, provides the foundation for plant regeneration by tissue culture. In order to manipulate plants for better crop varieties, plant cell and tissue cultures are crucial. In order to develop mulberry plants using micropropagation and molecular methods, plant regeneration systems are crucial (Chakraborty *et al*., 2015). Additionally, in vitro screening of genotypes for stress tolerance and yield, production of haploids and triploids, and improve ment of secondary metabolites through transgenic approach are recommended. A type of woody tree is the mulberry. Although a number of procedures have been established thus far for a time-phased screening strategy to transform *Morus indica* L. utilizing *Agrobacterium tumefaciens* and particle bombardment, there are still several

challenges in doing so. Mulberries are very vulnerable to stressors like drought and salinity, and their leaf moisture content drastically decreases in response to these challenges.

Transgenic Silkworm:

In silkworm also, germline transformation of *Bombyx mori* L. using microinjection of *PiggyBac* (PB) dericed vectors and its optimization was elaborated extensively Tamura *et al*. (2000) have developed a transgenic silkworm using DNA transposon *PiggyBac* as a vector for inserting the target gene into the silkworm chromosome. Use of trans genic silkworms to produce different recombinant protein as well as for produce high quality silks employing silk-gland as bioreactor have also become a reality now a days. Recent years have also seen the continued development of transgenic silkworms for monoclonal antibody medications for cancer, orphan diseases, and animal medications. Thus, efforts to create genetically modified silkworms could result in numerous advancements in sericulture and sericology. Recent developments opening up new possibilities for transgenic research in sericulture include the production of recombinant human serum albumin (HSA) in transgenic cocoons and the production of glycoproteins with decreased antigenicity in transgenic silkworms.

Genomics, Transcriptomics, Proteomics, and Metabolomics:

The majority of silk moth species belong to the Lepidoptera order's Bombycidae family. There are around 160,000 species in this family, most of which are agricultural pests. Comparative genomics and functional genomics are just two of the many subfields that make up genomics. These genetic elements serve a

variety of purposes in the development of silkworms. Chinese academics from Southwest University in Chongqing, China, and Japanese researchers from the University of Tokyo worked together to sequence the first silkworm's (*Bombyx mori*) genome. The development of high-throughput sequencing technologies has revolutionized sericulture research by enabling comprehensive analysis of silkworm and mulberry genomes. Whole-genome sequencing has identified thousands of genes responsible for silk gland development, immunity, metabolism, growth regulation, and stress responses. Transcriptomic analysis using RNA sequencing (RNA-Seq) has improved understanding of gene expression during different developmental stages of *Bombyx mori*. It provides valuable insights into the molecular mechanisms regulating cocoon formation, silk protein synthesis, metamorphosis, pathogen infection, and environmental adaptation. Proteomics complements transcriptomic studies by identifying proteins actively involved in biological processes. Analysis of silk gland proteins has revealed enzymes responsible for fibroin and sericin biosynthesis, while immune proteomics has identified proteins associated with pathogen defense mechanisms. These discoveries facilitate the development of disease-resistant silkworm breeds and improve silk quality. Comparative integument tran scriptome analysis identified genes associated in the pattern formation in three allelic mutants of B. mori and eight common genes involved in the construction of multiple twin- spot marking patterns in the three mutants (Ding *et al*., 2020).

Table 1: List of Molecular markers used in Silkworm *Bombyx mori* L.

Marker type	Silkworm races/strain	Country	Remark	References
RAPD	NB1, N124.C124, C108, Boropolu,Hu204, Chinese hybrid, Chinese Golden-70, Nan Naung 6A	India	Genetic diversity	Chatterjee *et al*. (2003)
ISSR	TMS-2, TMS-12, TMS-14, TMS-17, ODT	India	Genetic relationship	Velu *et al*. (2008)
SSR	Pure Mysore, ND7, Nistari, L14	India	Genetic diversity	Chandrakanth *et al*. (2014)
SSR	Zhenze, Lu108, Zhong20xi, Yanhe No. 1	China	Genetic Fingerprint	Qian *et al*. (2019)
AFLP	Khorasan Lemon, Khorasan Orange	Iran	Genetic diversity	Dalirsefat et al. (2007)

DNA Markers:

DNA markers are now essential instruments for investigating gene-related research, including genotype screening, gene identification, and germplasm collection characterization. Single nucleotide polymorphisms (SNPs), inter simple sequence repeats (ISSRs), simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs), and restriction fragment length polymorphisms (RFLPs) are among the variety of DNA markers that offer distinct benefits for genetic analysis (Lusser *et al*., 2012). Marker-assisted selection (MAS) offers a number of advantages over traditional breeding techniques that significantly improve the breeding process.

Clustered Regularly Interspaced Palindromic Repeats (CRISPR/Cas) System:

The CRISPR/Cas9 system is based on a bacterial immune system in which Cas9 is guided to its specific target DNA by CRISPR RNAs (crRNAs) and the trans- activating CRISPR RNA (tracrRNA). The experimental method mainly involves the introduction of muta tions into the target DNA region through the injection of single-guide RNA (sgRNA), which is RNA fused with crRNA and tracrRNA, and the Cas9 protein into the eggs immediately after they are laid. The injection of just two reagents, sgRNA and Cas9 protein, induces the cleavage of the genomic DNA cor responding to the target sequence. A novel gene-editing method known as CRISPR-Cas9 allows precise modification of an organism's DNA. It is composed of two main components: the Cas9 protein, which acts as a molecular scissors, and a guide RNA (gRNA), which points the Cas9 protein toward the target DNA sequence. The Cas9 protein then breaks DNA, and when the cell repairs these breaks, it may lead to alterations in genes. According to Schuijff *et al*. (2021), these methods also advance our knowledge of gene functions and the mechanisms behind a variety of illnesses and hereditary disorders. In the field of genome editing, it is essential to minimize off-target consequences.

RNA Interference (RNAi) Technology:

RNA interference (RNAi) is a naturally occurring gene-silencing mechanism that has become an important tool for functional genomics and disease management in sericulture. RNAi selectively suppresses the expression of target genes by degrading complementary messenger RNA (mRNA), thereby preventing protein synthesis. In sericulture, RNAi has been widely employed to investigate gene function related to immunity, development, metabolism, reproduction, and silk protein synthesis. It also provides an environmentally friendly approach for controlling insect pests and microbial

pathogens without relying heavily on chemical pesticides.

Conclusion:

Traditional sericulture involves breeding of silkworm for production of silk that provides livelihood chance to millions of people in the country alongside generating foreign currencies. In light of the current situation, it is important to diversify the entire sericulture process in order to meaningfully realize its yield. A new field of synthetic science for the manufacturing of silk has been made possible by the development of contemporary biotechnology and its applications. The adoption of contemporary biotechnology techniques, such as marker-assisted selection and transgenic production of foreign proteins, can successfully harness the enormous potential of the silk industry.

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