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## OVERVIEW OF BIOTECHNOLOGICAL INTERVENTIONS IN TASAR SERICULTURE: PROSPECTS FOR BRIDGING TRADITION TO TECHNOLOGY

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### ABSTRACT

Tasar Sericulture, as a science, significantly contributes to employment opportunities, generating livelihoods for approximately 3.5 lakhs peoples of India. Various biotechnological studies conducted in the field of tasar sericulture. This comprehensive review delves into the various aspects of tasar sericulture, with a specific focus on *Antheraea mylitta*, the tasar silkworm, and explores the application of biotechnological approaches to improve its utility. Biotechnology emerges as a pivotal domain, offering innovative solutions to enhance sericulture productivity. The potential of biotechnological interventions, particularly in genetic engineering and molecular biology, is highlighted for optimizing processes, increasing yields, and overcoming traditional challenges. Considering the evolving climate patterns and challenges faced by sericulture, there is an imperative need to transition from traditional methods to advanced scientific knowledge. While the molecular study of tasar silkworms needs a comprehensive information, there is substantial potential to harness biotechnological tools for conservation and breed improvement. Traditional gel-based techniques have paved the way for modern high-throughput approaches, including Allele-specific hybridization, single-base primer extensions, oligonucleotide ligations, and DNA arrays. Advancements in Next-Generation Sequencing (NGS) technology, such as whole genome sequencing and reduced representation sequencing, offer new avenues for comprehensive exploration of the tasar silkworm's genetic makeup.

**Keywords:** *Antheraea mylitta*, Characterization, Conservation, Markers, Sericulture.

### Introduction

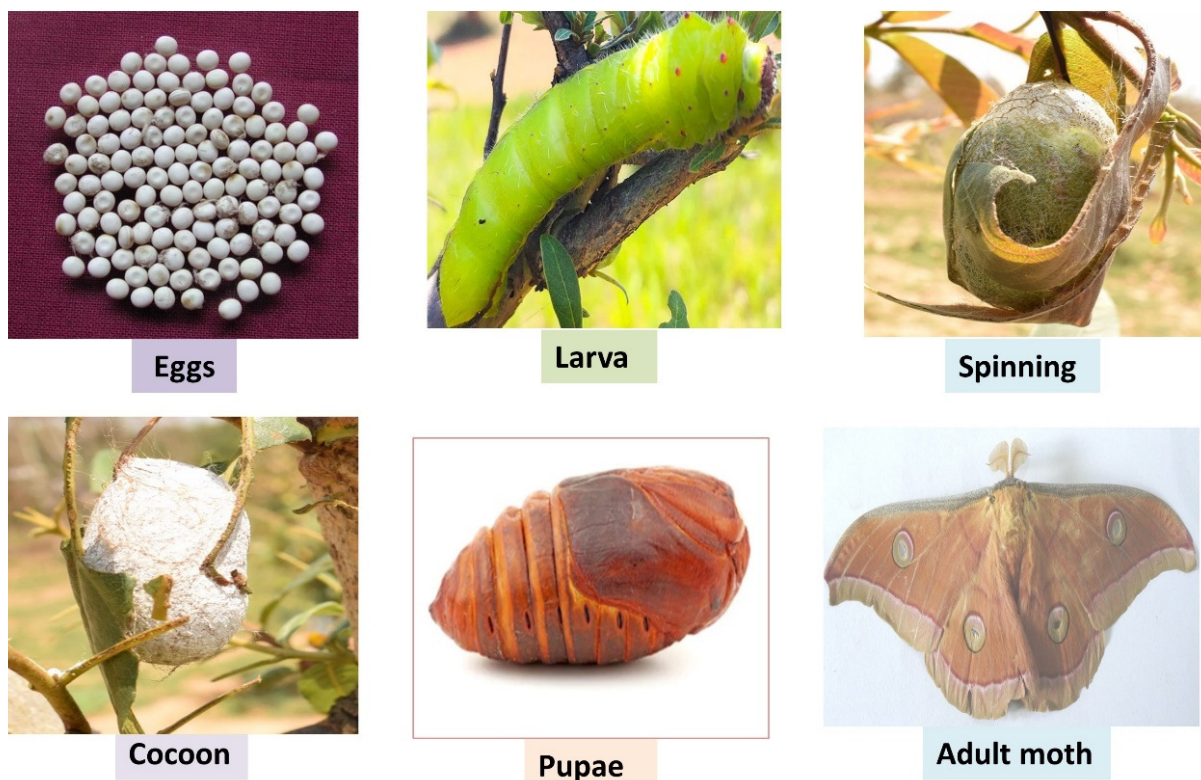
Biotechnology emerges as a pivotal domain, offering innovative solutions to enhance sericulture productivity. The potential of biotechnological interventions, particularly in genetic engineering and molecular biology, is highlighted for optimizing processes, increasing yields, and overcoming traditional challenges. Sericulture, the comprehensive science of synthesizing silk, generates huge employment opportunities for throughout the country, encompassing the entire value chain from soil cultivation to silk production (Bhattarcharjya *et al.*, 2020). Silk stands out as one of the most valuable natural fibers, with the approximate unit price of raw silk being estimated to be around twenty percent higher than that of raw cotton (Masig *et al.*, 2019). Tasar silk

can be traced back to literature dating as far as 1590 B.C., with the term "tasar" believed to have originated from the Sanskrit word "trasara" (Shuttle). Tasar silkworm, eri-silkworm, oak-tasar silkworm, and muga silkworm are among the wild silkmths. The majority of research and technological development in this field is primarily concentrated in Asia, with China, India, and Japan leading the way (Bukhari *et al.*, 2019). Tasar silkworm, scientifically known as *Antheraea mylitta*, is a silk-producing moth native to tropical India. It is a member of the *Saturniidae* family and is widely recognized for its role in sericulture and silk cultivation. Tasar silkworms have specific host plants on which they feed. The most common host plants for tasar silkworms include various species of *Terminalia* and *Shorea*, which are trees found in the natural habitat of these moths (Bhatia and Yousuf, 2014). Tasar silk

production is prominent in several states of India, including Jharkhand, Chhattisgarh, Bihar, Odisha, Maharashtra, and some parts of Madhya Pradesh (Ojha and Panday, 2004). The tasar silk fiber is characterized by its unique colour and coarse texture. Yet, it exhibits superior tensile strength, elongation, and stress-relaxation values when compared to the mulberry silk fiber produced by the *Bombyx mori* silkworm (Iizuka, 2000).

The Indian tasar silkworm, *A. mylitta*, naturally occurs in the tropical regions of India and is found in various geographical locations and habitats across the country. Due to the unique ecological conditions present in these diverse areas, multiple morphological variants, traditionally referred to as ecoraces, have been recognized in *A. mylitta* (Jolly *et al.*, 1974). Each region may have its ecoraces adapted to local conditions. The specific ecoraces and their distribution can be influenced by factors like climate, altitude, and the types of host plants available in a particular area (Wang *et al.*, 2009). A total of 44 eco-races have been documented in the tasar silkworm species. These

ecoraces, categorized as uni-, bi-, or trivoltine, vary based on geo-ecological conditions and exhibit distinctions in various qualitative and quantitative traits, including cocoon weight and color, as well as larval color (Srivastava *et al.*, 1974). While the majority of these ecotypes do not naturally interbreed, certain ones have been observed to produce offspring when intentionally mated in captivity. In Figure 1, we have described all the activities from seed, larva, cocoon, pupa, and adult moth in Tasar silkworm along with spinning activity. Unlike mulberry sericulture, the cultivation of Tasar involves utilizing host plants in natural forest conditions, eliminating the need for investments in planting host plants, constructing rearing houses, and acquiring appliances. Tasar cultivation not only contributes to the preservation of forests but also provides an opportunity to harness extensive natural resources with minimal investment, while simultaneously generating substantial employment for rural tribal communities (Vishaka *et al.*, 2020).



**Fig. 1:** Lifecycle of *Antheraea mylitta*: Depiction of various stages

Due to evolving climate patterns and the intricate challenges faced by sericulture farmers, there is a necessity to transition from conventional approaches to the implementation of advanced scientific knowledge.

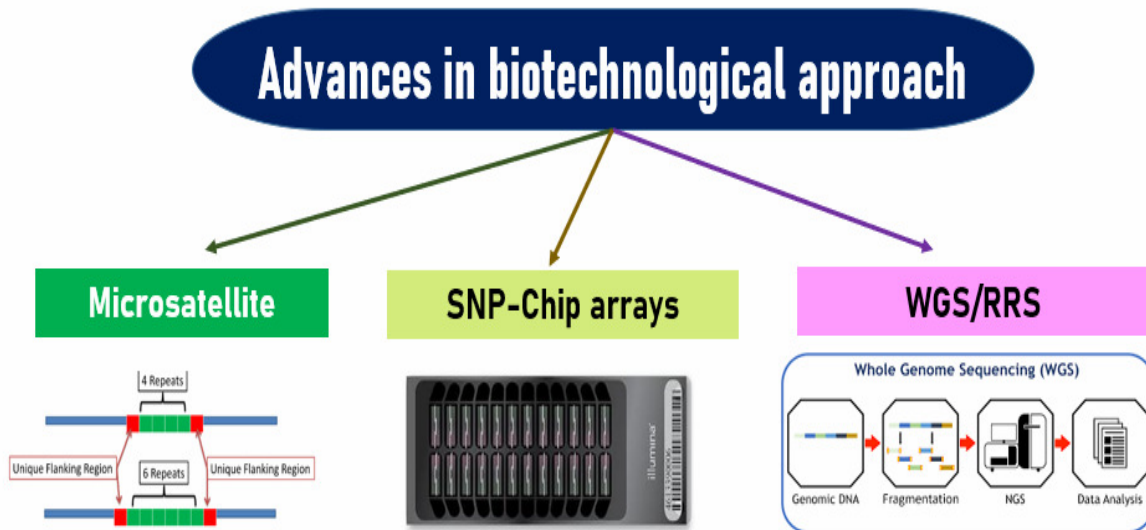
Presently, the potential and applicability of biotechnology in boosting sericulture productivity have been highlighted in numerous scientific talks (Alam *et al.*, 2022). Biotechnology stands as one of the forefront

domains in scientific advancement. The term can broadly be defined as the technology by which one can produce useful products from raw materials with the help of living organisms or other biological processes. Various biotechnological interventions can be employed to enhance production across different industries. Utilizing the capabilities of genetic engineering, molecular biology, and other cutting-edge techniques, industries can fine-tune their processes, optimize yields, and overcome challenges associated with traditional methods (Gupta and Savalia, 2012). In this review, we tried to comprise the different studies on the applications of biotechnological approaches in the improvement of tasar silkworms.

**Biotechnology approaches and tasar silkworm:**

In recent times, advancements in molecular biology and technology have led to the utilization of genetic markers to enhance overall production and improvement in different fields (Dekkers and Hospital, 2002). These genetic molecular markers are powerful tools for studying genetic diversity, relatedness, and disease susceptibility. The choice of marker type depends on the specific research and available resources. One traditional method for identifying important genes for genetic improvement is Restriction Fragment Length Polymorphism (RFLP), which

involves appropriately labeled probes. Hypervariable repetitive DNA sequences, such as microsatellites and minisatellites, can also be utilized for DNA fingerprinting by subjecting them to hybridization to reveal unique patterns (Deb, 2012). In the past decade, Single nucleotide polymorphisms have become increasingly popular despite being a biallelic marker type. They are currently the most abundant marker in animal and plant genomes. Besides being abundant, SNPs have garnered attention for their genetic stability and suitability for high-throughput automated analysis (Heaton *et al.*, 2002). SNP chips are susceptible to ascertainment bias due to their design, which frequently relies on SNPs that have already been identified and studied in specific populations. To address potential ascertainment bias when genotyping local breeds, advancements in next-generation sequencing (NGS) technology have led to the development of whole genome sequencing (WGS) and Reduced Representation sequencing (RRS) methods of genome resequencing. RRLs simplify genome complexity by enrichment, separation, and elimination prior to sequencing. RRS technologies, like RADseq, ddRAD, 2b-RAD, and GBS, rely on DNA digestion with restriction enzymes to decrease genome complexity (Wang *et al.*, 2020) (Figure 2).



**Fig. 2:** Trends in biotechnological approaches for improvement in different fields

**Identification of Repetitive DNA Sequences in the *A. mylitta*:**

Mahendran *et al.* (2005) investigated the characterization of a repetitive genomic DNA fragment using TaqI as a genetic marker to assess genetic variability and phylogeny between various eco-races. Analysis of the sequence and Southern blotting

validated the repetitive character of the TaqI DNA fragment, recognized as the *A. mylitta* TaqI family repeat, AmTFR. PCR amplification of AmTFR demonstrated its presence in all tested eco-races of the tasar silkworm, as well as in certain other silk-producing insects.

### Characterization of the Long Terminal Repeat Retrotransposon (Pao-like), Tamy, in *A. mylitta*:

Using a PCR-amplified partial fibroin gene sequence as a probe, Ghosh and co-worker (2005) identified a long terminal repeat (LTR) retrotransposon named Tamy in the *Antheraea mylitta* sub-genomic DNA library. Isolation of genomic DNA from the posterior silk glands of *A. mylitta* facilitated the characterization of Tamy, which spans 8387 nucleotides and possesses 1305 nucleotides of extended long terminal repeats at its 5' and 3'-ends (a significant feature indicative of a long terminal repeat retrotransposon).

### Phylogenetic Analysis of Silk-Producing Insects Using 16S Ribosomal RNA Sequences:

In 2006, Mahendran *et al.* conducted a phylogenetic investigation involving *Antheraea mylitta* and 13 additional silkworm species, relying on sequences derived from the 16S ribosomal RNA (rRNA) and cytochrome oxidase subunit I (coxI) genes. They utilized the maximum likelihood and maximum parsimony methods. The weighted parsimony analysis (where emphasis was placed on transversions relative to transitions (ts, tv4) for coxI) was found to be more robust compared to unweighted parsimony. This approach also favoured the tree topology observed in the 16S rRNA analysis.

### Next-Generation Sequencing (NGS) for Genetic Insights into the Population of Indian Tropical Tasar Silkworm (*A. mylitta*):

Analysis of the sequencing library revealed fragment sizes ranging from 200bp to 700bp, identifying a total of 35,877 sites across 8 samples. Subsequent phylogenetic analysis elucidated both closely and distantly related taxa among the populations, providing valuable insights into their genetic relationships. Complete genome sequencing of *Antheraea mylitta* was performed at the Central Tasar Training and Research Institute and submitted to NCBI with GenBank assembly GCA\_014332785.1 (Pandey *et al.*, 2020). With a genome size of 698.4 Mb, a total ungapped length of 658.6 Mb, containing 16,774 scaffolds. Central Tasar Training and Research Institute and Central Silk Board sequenced the genome in different ecoraces of *Antheraea mylitta* using different platforms. These sequences have been submitted to NCBI GenBank with the following reference IDs SRX8220370, SRX8220369, SRX7551690, SRX7551647, and SRX6849305 (Table 1). Next-generation sequencing technologies, alongside *in silico* analysis are modern sequencing methods employed in population genetic studies to explore the evolutionary forces impacting genetic variation. In their study, Gattu *et al.* (2024) utilized genomic DNA from parental ecoraces – Andhra local and Daba TV – as well as their hybrid populations, which were independently sequenced using the Illumina NextSeq500 platform.

**Table 1:** Summary of Sequencing Approaches, NCBI Accession Numbers, and Platforms Used in *Antheraea mylitta*.

Sequences	NCBI accession number	Platform	Institute
Sequencing of the genome of Tasar silkworm, <i>Antheraea mylitta</i>	SRX8220370	PacBio_SMRT	CTR&TI
Sequencing of the genome of Tasar silkworm, <i>Antheraea mylitta</i>	SRX8220369	Illumina (HiSeq X Ten)	CTR&TI Ranchi
16S rRNA-seq of <i>Antheraea mylitta</i> race	SRX7551690	Illumina (HiSeq 2500)	Central Silk Board
16S rRNA-seq of <i>Antheraea mylitta</i> modal ecorace	SRX7551647	Illumina (HiSeq 2500)	Central Silk Board
Metagenomic data of midgut microbiota of Tasar silkworm	SRX684930	Illumina (HiSeq 2500)	Central Silk Board

### Utilisation of Different Types of Markers

Any enduring and heritable variable that can be quantified or identified through an appropriate method and subsequently employed to identify the presence of a specific genotype or phenotype other than the variation itself, which otherwise is non-measurable or very difficult to detect is depicted as a marker (Taylor

and Lewontin, 2017). Genetic markers can be categorized into three classes: morphological markers, reflecting variations at the phenotype level; biochemical markers, indicating variations at the gene product level; and molecular markers, representing variations at the DNA level (Patwardhan *et al.*, 2014).



Molecular markers are designed to identify variations in DNA that can serve as diagnostics for a species, genotype, or variety. A molecular marker can be used to detect variations in the DNA sequence. These markers expedite the estimation of genetic diversity and relatedness in germplasm, and they enhance the accuracy of establishing phylogenetic relationships compared to previous methods involving morphological and biochemical techniques (Jiang, 2013). The classification of molecular markers can vary depending on the technique employed for detection and amplification. Restriction Fragment Length Polymorphism (RFLP) relies on changes in restriction sites within the target DNA, followed by hybridization with probe DNA. Random Amplified

Polymorphic DNA (RAPD), Sequence Characterized Amplified Region (SCAR), and Sequence Tagged Sites (STS) are based on mutations at the primer annealing site in the target DNA. Cleaved Amplified Polymorphic Sequence (CAPS) and Amplified Fragment Length Polymorphism (AFLP) involve both restriction site changes and mutations at the primer annealing site in the target DNA. Additionally, Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), and Single Nucleotide Polymorphism (SNP) markers exist (Table 2). Different researchers have periodically utilized these markers to study various ecoraces, a discussion of which will follow in the next section.

**Table 2:** Utilization of molecular markers for the various applications in Tasar silkworm (*A. Mylitta*)

S. No.	Research	Year	Reference
1	Analysis of inheritance variability in <i>Antheraea mylitta</i> ecotypes using SSR profiling	2004	(Chatterjee <i>et al.</i> )
2	Study of genetic variability and population structure of wild and semi-domesticated populations of <i>Antheraea mylitta</i> ecotypes using SSR markers	2005	(Kar <i>et al.</i> )
3	Identification of <i>Antheraea mylitta</i> ecotypes using RAPD and SCAR markers	2006	(Saha and Kundu)
4	Study of repetitive DNA in thermophilic silkworm <i>Antheraea mylitta</i>	2006	(Mahendran <i>et al.</i> )
5	Analysis of genetic diversity in environmental populations of thermophilic silkworm <i>Antheraea mylitta</i> using RFLP technique	2006	(Mahendran <i>et al.</i> )
6	Development of advanced DNA markers for thermophilic silkworm <i>Antheraea mylitta</i>	2008	(Saha <i>et al.</i> )
7	Identification of RAPD and SCAR markers associated with productivity traits in thermophilic silkworm <i>Antheraea mylitta</i>	2012	(Datta <i>et al.</i> )
8	Genetic analysis of the Indian tasar silkworm ( <i>Antheraea mylitta</i> ) population	2015	(Chakravarti <i>et al.</i> )
9	Development of molecular markers for the selection of thermo-tolerant lines in tasar silkworm and their use in selection	2023	(Prabhu <i>et al.</i> )

Recent advancements in molecular biology have equipped researchers in biotechnologies with a diverse set of new tools, enabling them to tackle issues spanning from gene expression to genetic diversity. The silkworm itself has been extensively utilized as a model insect in basic research within the fields of biotechnology and molecular genetics (Buhroo *et al.*, 2019). Scientists in sericulture are adeptly employing various markers due to their crucial role in the conservation study of silkworm genetic fingerprinting, variability, diversity, and relationships. These markers are essential for creating linkage maps and identifying people or lines carrying specific genes.

**Utilization of RAPD and SCAR Markers for identification of *A. mylitta* Ecoraces:** Saha and Kundu (2006) employed ten ecoraces of *Antheraea mylitta*, commercially exploited for tasar silk production. To complement existing morphological markers, they utilized DNA markers such as Random Amplification of Polymorphic DNA (RAPD) and Sequence-Characterized Amplified Region (SCAR). Seven RAPD bands were selected, identifying eight

out of the ten ecoraces. Sequencing of these identified RAPD fragments led to the design of primers for SCAR markers. Among the seven sets of primers, one pair produced polymorphic SCAR bands that could diagnose five of the ten ecoraces. The combined use of both markers enabled the identification of all ten ecoraces (Saha and Kundu, 2006). Dutta *et al.* (2012) identified a randomly amplified polymorphic DNA (RAPD) marker, investigated its inheritance, and concurrently developed a stable diagnostic sequence-characterized amplified region (SCAR) marker by segregating the silkworm into groups with high cocoon and shell weights (HCSW) and low cocoon and shell weights (LCSW). These markers can aid in selecting the optimal parental stock of tasar silkworms for achieving HCSW in breeding programs. Prabhu *et al.* (2023) developed markers using a combination of random amplified polymorphic DNA (RAPD) and sequence-characterized amplified region (SCAR) techniques to aid in the selection of a thermo-tolerant line of the tropical Tasar silkworm. The TT-PB1

exhibited greater specificity in identifying the thermo-tolerant line of *A. mylitta*.

**Utilization of RFLP Technique to Assess Genetic Variability in Ecoraces of the *A. mylitta*:** Mahendran *et al.* (2006) successfully characterized and cloned a 281 bp Mbol fragment of genomic DNA. This fragment exhibits a 75% identity at the protein level with the 'reverse transcriptase' site of the TED retrotransposon found in the lepidopteran insect *Trichoplusia ni*. Despite this, studies employing RFLP and utilizing the Mbol fragment as a probe revealed polymorphic patterns among the ecoraces. The obtained kinship structure of different ecoraces, based on RFLP patterns, aligns with both phenotypic and geographical correlations.

**Random Amplified Polymorphic DNA (RAPD) Markers for the *A. mylitta*:** Saha *et al.* (2008) employed the RAPD method to assess genetic variability among different eco-races. A total of 80 random ten nucleotide length primers were utilized for RAPD amplification, resulting in 415 reproducible bands. These bands were then employed to generate a Reynold distance and for subsequent clusters utilizing the unweighted pair-group method with an arithmetic average. The number of polymorphic bands detected by each primer ranged from 5 to 24, with an average of 14.1 per primer. Genetic distance values ranged from a minimum of 0.0108 between Modal and Nalia ecoraces to a maximum of 0.0244 between Modal and Andhra local. The observed polymorphism percentage was 81.9%.

**Use of ISSR markers for Genetic structure of *A. mylitta*:** Kar *et al.* (2005) ISSR markers across 56 individuals from three populations, achieving a remarkable 98% polymorphism. The individual populations exhibited percentage polymorphism rates of 58.69% for semi-domestic bivoltine (DB), 52.9% for trivoltine (DT), and 77.54% for naturally grown wild populations (DN). Chatterjee *et al.* (2004) utilized ISSR profiling to analyze the genetic variability within the ecotypes of *Antheraea mylitta*, the tropical Tasar silkworm. The findings derived from the analysis of polymorphism, revealed by twelve ISSR primers, encompassed 11 populations of *A. mylitta*, representing six eco-races, along with 41 individuals of the Raily collected from different distinct areas of the Dandakarnya forest in Madhya Pradesh. Devi *et al.* (2012) unveiled the genetic diversity among the Indian Oak Tasar Silkworm, *Antheraea proylei* utilizing ISSR markers. Srivastava *et al.* (2008) evaluated genetic diversity in various populations of the Raily ecorace of *Antheraea mylitta* utilizing ISSR markers. Vijayan *et al.* (2005) conducted a study on three ecoraces (Raily,

Daba, and Modal) utilizing 12 ISSR and 10 RAPD primers. The observed significantly low genetic differentiation ( $G_{ST}$ ) values and the high gene flow (Nm) among the ecoraces suggest that the level of genetic diversity present among them is not substantial enough to prompt significant genetic drifts shortly.

**Utilization of microsatellite markers for genetic study of *A. mylitta* populations:** Chakraborty *et al.* (2015) constructed microsatellite markers for *A. mylitta*. The screening was performed at each locus exploiting a total of 154 moths from eight distinct ecoraces. Hierarchical analysis of population structure, employing Analysis of MOlecular VAriance (AMOVA), uncovered notable structuring ( $F_{ST} = 0.154$ ) and significant inbreeding ( $F_{IS} = 0.505$ ). Renuka and Shamitha (2016) identified the genetic diversity among these ecoraces using DNA markers, specifically simple sequence repeats (SSRs), the majority of which generated polymorphic bands.

#### **Other allied applications of biotechnology in the tasar sericulture**

Apart from the direct characterization and diversity analysis in Tropical tasar silkworms, Biotechnological interventions have been utilized in different aspects also like cocoonase characterization, anti-microbial peptide in Haemolymph, the fibroin gene differential expression, the role of transferrin in the silkworm for antioxidant defense, etc.

**Characterization of *A. mylitta* cocoonase:** Sneha *et al.* (2022) conducted an extensive characterization of the cocoonase enzyme, also recognized as serine-trypsin protease or trypsin-like protease, utilizing various computational tools (Felsted *et al.*, 1973). These tools included ProtParam, Iterative Threading Assembly Refinement (I-TASSER), PROCHECK, SAVES v6.0, TM-align, Molecular Evolutionary Genetics Analysis (MEGA) X, and Figtree. The in-silico investigation of *A. mylitta* cocoonase revealed a 26% similarity with Qing-6 cocoonase of *A. pernyi* strain through Blastp analysis. Furthermore, it was identified as a chymotrypsin-like serine protease superfamily member.

**Characterizing a newly discovered antimicrobial peptide (QAK) derived from the *A. mylitta* hemolymph:** Chowdhury *et al.* (2020) identified various defense molecules, including antimicrobial peptides (AMPs) and proteins, from *Antheraea mylitta*. Through sequential chromatographic separation procedures, they Extracted and refined one antimicrobial tri-peptide. The peptide's amino acid sequence was determined as NH<sub>2</sub>-Gln-Ala-Lys-COOH (QAK) using MALDI MS/MS fragmentation analysis.

Subsequently, the peptide was synthesized in vitro using solid-phase peptide synthesis chemistry and acetylated through an acetic anhydride reaction. The antimicrobial activities of both non-acetylated and acetylated QAK were then tested against *Escherichia coli* and *Staphylococcus aureus* bacteria.

**Differential expression of fibroin gene in *A. mylitta*:**

Dutta *et al.* (2001) conducted a study on the differential analysis of the fibroin-producing gene across different developmental stages of the silkworm, *Antheraea mylitta*. They used genomic DNA extracted from each larval developmental stage, employing the labeled fibroin gene to conduct Dot blot hybridization. The results confirmed that the fibroin gene's relative concentration remained constant across all developmental stages at the genomic level. Using Northern hybridization and the fibroin gene as a probe, total RNA from the larval silk gland was analyzed, and it was revealed that fibroin expression occurs during intermoult stages and is repressed during moulting stages. Additionally, Western blot analysis of fibroin protein production, using anti-fibroin antibody, affirmed the differential expression of fibroin, consistent with fibroin mRNA synthesis.

**Role of transferrin in the silkworm for antioxidant defense:**

Dutta *et al.* (2019) presented the inaugural report on transferrin in the tropical tasar silkworm revealing

its potential involvement in antioxidant defense. This insight was derived from a comprehensive analysis involving proteomics and immunodetection techniques. Utilizing SDS-PAGE coupled with LC-MS/MS, we identified a 75.7 kDa protein in advanced larvae of *A. mylitta* as transferrin (AmTsf). The developed antibody facilitated the determination of its tissue-specific expression and functional relevance during development. This study contributes to the understanding of transferrin's diverse roles in insects and sheds light on the relatively unexplored area of iron-associated H<sub>2</sub>O<sub>2</sub> metabolism and redox homeostasis, particularly in the absence of catalase and glutathione peroxidase. In Figure 3 we have included all the available applications of biotechnology in tasar sericulture.

**Diapause-Specific Expressed Sequence Tags in *A. mylitta* (Drury):**

The tropical tasar silkworm *Antheraea mylitta* Drury, specifically the Daba bivoltine ecorace, exhibits facultative pupal diapause and displays various forms of voltinism. Mishra *et al.* (2011) have documented the identification of diapause-specific expressed sequence tags (ESTs) derived from PCR clones of genes such as Hsp70, Hsp23, hexamerins, and PCNA.

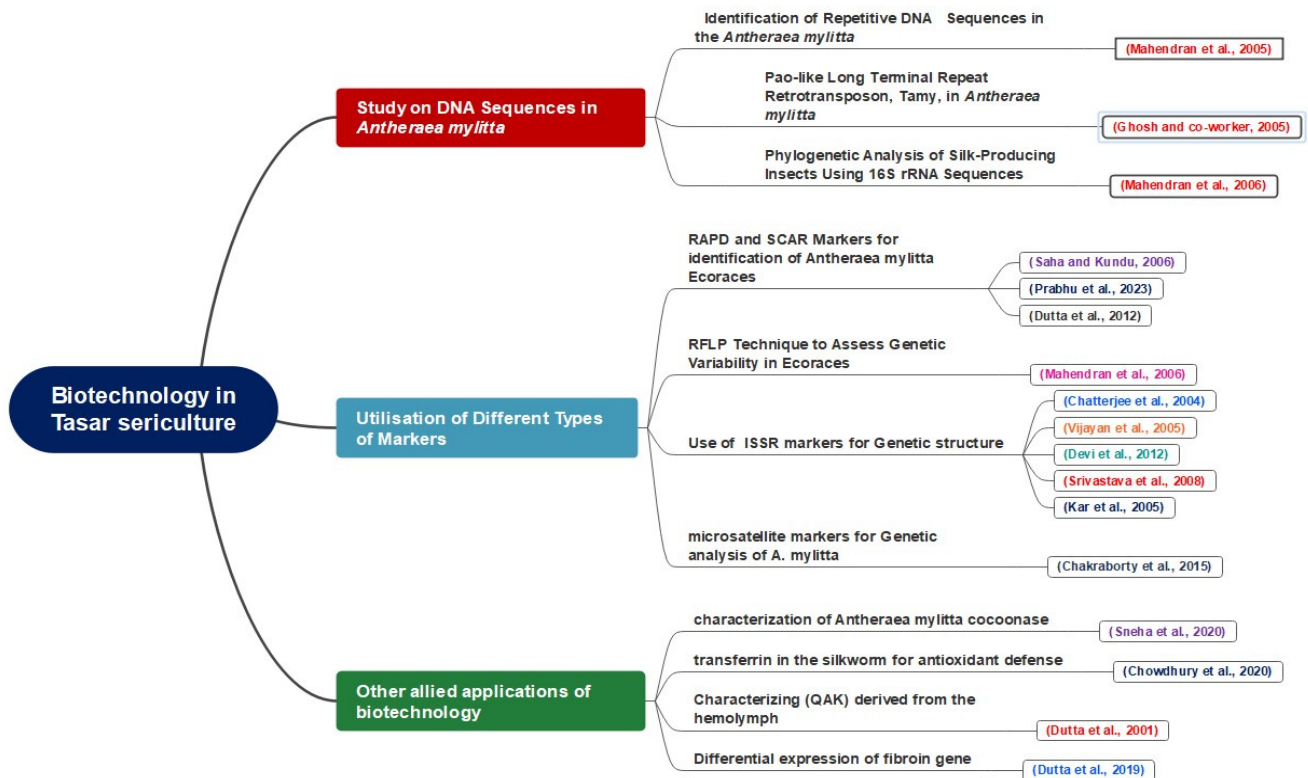


Fig. 3: Application of biotechnology in the field of tasar sericulture

## Future Prospects

India, the world's second-largest producer of raw silk after China. Tasar production in India's needs more attention. In order to enhance Tasar silk production, there is an urgent need to utilize advanced biotechnological approaches such as PCR-based DNA marker technology (Heywood and Dulloo, 2005). In addition to conventional preservation methods for significant ecoraces, the thorough characterization of diverse ecoraces and the assessment of their genetic diversity offer valuable insights that can inform conservation strategies. The application of biotechnological tools in the conservation of different tasar silk ecoraces provides researchers with precise and effective methods for preserving genetic diversity amid environmental challenges (Pathak *et al.*, 2014).

### Utilization of molecular markers

It is evident that the molecular study of tasar silkworms is still in its preliminary stages, with considerable potential for harnessing the application of biotechnological tools in tasar culture, particularly for conservation and breed improvement. Traditional techniques, such as gel-based methods, including Amplification of Refractory Mutation System (ARMS), RFLP, and AFLP, have paved the way for modern high-throughput approaches like Allele-specific hybridization, single-base primer extensions, oligonucleotide ligations, and DNA arrays (Mondini *et al.*, 2009; Jiang *et al.*, 2013).

### Exploitation of the next generation of sequencing

Identification, annotation and regulation of genes responsible for commercial character of tasar silkworm is the key area of research. Advancements in Next-Generation Sequencing (NGS) technology have ushered in whole genome sequencing (WGS) and reduced representation sequencing (RRS) (Heffelfinger *et al.*, 2014). These cutting-edge techniques open new avenues for comprehensive exploration and understanding of the tasar silkworm's genetic makeup. Looking ahead, the convergence of biotechnological innovations, genetic research, and conservation efforts will likely revolutionize tasar sericulture. The identification of novel genes, development of disease-resistant breeds, and synthesis of crucial biological molecules represent key areas where biotechnology can contribute significantly. Moreover, the conversion of sericulture waste into beneficial resources aligns with sustainable practices and environmental protection.

## Utilization of different modern biotechnological equipment:

Modern biotechnological equipment can be harnessed in tasar sericulture to boost efficiency and enhance the genetic quality of silk production, as discussed below (Figure 4).

- i. DNA Sequencers:** Employing high-throughput DNA sequencers for genetic analysis and marker-assisted selection in breeding programs.
- ii. PCR Machines:** Utilizing Polymerase Chain Reaction (PCR) machines for amplification of specific DNA sequences, aiding in genetic identification and characterization.
- iii. Next-Generation Sequencing (NGS) Platforms:** Leveraging NGS platforms for whole-genome sequencing, enabling comprehensive genomic analysis for trait selection and breeding.
- iv. Bioinformatics Tools:** Utilizing bioinformatics software and databases for analyzing large-scale genomic data, facilitating the identification of candidate genes and genetic markers associated with desirable traits.
- v. Automated Silk Reeling Machines:** Implementing automated silk reeling machines to enhance efficiency and productivity in the silk reeling process.
- vi. Genotyping Arrays:** Utilizing genotyping arrays for high-throughput genotyping of genetic variations, aiding in the identification of economically important traits in Tasar silk production.
- vii. Microscopes and Imaging Systems:** Employing advanced microscopy and imaging systems for detailed analysis of silk quality, including fiber diameter, texture, and color.
- viii. Bioreactors and Fermentation Equipment:** Utilizing bioreactors and fermentation equipment for the production of recombinant proteins or enzymes involved in silk production processes.
- ix. Gene Editing Tools (e.g., CRISPR/Cas9):** Utilizing gene editing tools for precise manipulation of silk-related genes to improve silk quality and yield.
- x. Automated Sorting and Grading Systems:** Implementing automated sorting and grading systems for efficient classification of silk cocoons based on quality parameters, optimizing silk production processes.





(PCR)



Nanodrop Spectrophotometer



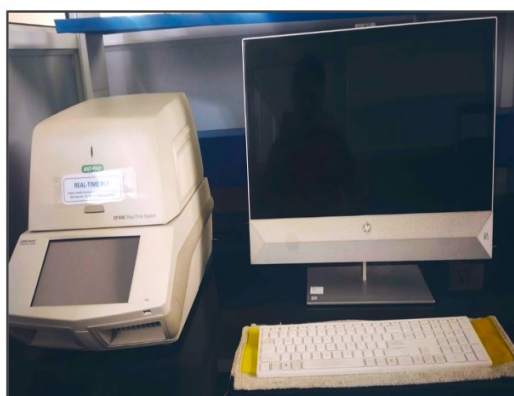
Lyophilizer



Kryo PLANER



Brightfield microscope



(Real-time PCR)



(Oxford nanopore sequencer)

**Fig. 4:** Different biotechnological equipments available at Central Tasar Research and Training Institute, Ranchi

## Conclusion

Tasar Sericulture contributes to employment opportunities, generating livelihoods for approximately 3.5 lakhs peoples of India. Various biotechnological studies conducted in the field of tasar sericulture has been documented to explore the application of biotechnological approaches to improve its utility in tasar silk sector. The incorporation of biotechnological advancements into tasar sericulture not only holds the potential to propel the industry to unprecedented heights but also ensures its resilience in the face of evolving environmental and economic challenges. The ongoing integration of cutting-edge technologies and methodologies underscores a promising future for tasar sericulture, where biotechnology plays a pivotal role in shaping sustainable practices and fostering innovation.

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