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## MOLECULAR INTERVENTIONS IN SERICULTURE: A FOCUS ON THE TROPICAL TASAR SILKWORM

Vikas Kumar<sup>1</sup>, Rahul Prasad<sup>1</sup>, Obaidullah Ehrar<sup>1</sup>, Kishore Kumar<sup>1</sup>, Nidhi Sukhija<sup>1</sup>, Manjappa<sup>2</sup>,  
Mohammed Muzerudin Baig<sup>1</sup>, Hanamant Gadad<sup>1</sup>, Jitendra Singh<sup>1</sup>, Jay Prakash Pandey<sup>1</sup>, N. B.  
Chowdary<sup>1</sup> and Immanuel Gilwax Prabhu<sup>1\*</sup>

<sup>1</sup>Central Tasar Research and Training Institute, Ranchi - 835303, Jharkhand, India

<sup>2</sup>Central Sericultural Research & Training Institute, Mysuru - 570008, Karnataka, India

\*Corresponding author: [immanuel.gilwish@gmail.com](mailto:immanuel.gilwish@gmail.com)

### ABSTRACT

The Tasar silkworm (*Antheraea mylitta*) plays a pivotal role in the sericulture industry, particularly in India, where it has been cultivated for centuries. Its adaptability to diverse climatic conditions enhances its suitability for sericulture in a range of geographic locations. This review explores the physiological and molecular responses of Tasar silkworms to environmental stress, especially temperature-induced oxidative stress, which can generate reactive oxygen species (ROS) and cause significant damage to cellular components. The study of antioxidant defense mechanisms in these silkworms, including both enzymatic and non-enzymatic pathways, provides crucial insights into their resilience to oxidative damage. Furthermore, the role of heat shock proteins (HSPs) in thermal stress response is emphasized, highlighting the differential expression and regulation of HSPs such as Hsp21 and Hsp70. These proteins act as molecular chaperones, protecting cellular proteins from denaturation under elevated temperatures. Comparative studies of HSP expression across various silkworm strains and developmental stages underscore the complexity of the thermal stress response, aiding in the identification of genetic factors that contribute to thermotolerance. Notably, recent advancements, such as the development of the SCAR marker TT-PB1, have revolutionized the analysis of thermotolerance inheritance, paving the way for targeted breeding strategies. Molecular breeding, particularly Marker-Assisted Selection (MAS), is highlighted as a powerful tool for enhancing silk yield and improving desirable traits in silkworms. The integration of traditional breeding methods with molecular insights from genomic databases like SilkDB and KAIKObase enhances our understanding of genetic diversity and facilitates the development of superior silkworm strains. In addition, transgenic technology offers promising avenues for gene manipulation, enabling the introduction of foreign genes to improve silk quality, tensile strength, and disease resistance. This review also delves into the molecular mechanisms underlying the pupal diapause phenomenon in Tasar silkworms, focusing on the identification of diapause-specific expressed sequence tags (ESTs) associated with genes such as Hsp23, and hexamerins. Understanding these molecular aspects provides valuable insights for managing diapause and optimizing silk production. The utilization of molecular markers, including RAPD, ISSR, and SNPs, has significantly advanced our knowledge of genetic diversity and phylogenetic relationships among silkworm strains. These markers are instrumental in deciphering genetic closeness, identifying wild ecoraces, and enhancing disease resistance through techniques like RNA interference (RNAi). *In silico* approaches, such as whole-genome shotgun sequencing (WGS), next-generation sequencing (NGS), and RNA sequencing (RNA-seq), are crucial for identifying genes associated with silk production and exploring genetic diversity. Additionally, CRISPR-Cas9 technology holds promise for precise gene editing, potentially transforming the silk industry by enhancing silk properties and production.

Overall, this comprehensive review underscores the importance of integrating molecular insights with traditional breeding methods to advance the Tasar silk industry, contributing to sustainable sericulture practices and the development of innovative silkworm strains for improved silk yield and quality.

**Keywords:** *In-silico* approaches, molecular marker, Tasar silkworm, heat shock proteins, and sequencing

## Introduction

Molecular interventions have significantly advanced sericulture, particularly in the context of the tropical Tasar silkworm, *Antheraea mylitta*. This review focuses on the molecular strategies that have been developed to enhance silk yield, improve thermal tolerance, and boost disease resistance in Tasar silkworms. Understanding the molecular mechanisms underlying silk production is essential for improving silk yield in silkworms. RNA sequencing (RNA-seq) has been pivotal in identifying genetic factors that influence silk yield. By developing markers associated with high silk yield, researchers can facilitate marker-assisted selection and targeted breeding programs. Utilizing genetic resources from diverse strains and comprehensive databases like SilkDB and KAIKObase, researchers can enhance breeding efforts tailored to specific environmental conditions. Traditional breeding methods are being complemented by modern techniques such as genetic engineering and biotechnological advancements, with Marker-Assisted Selection (MAS) proving to be a powerful tool in accelerating the breeding process and enhancing selection efficiency.

Thermal tolerance is crucial for the survival and productivity of silkworms in varying environmental conditions. Polyvoltine strains generally show better thermal tolerance compared to bivoltine strains. Research has revealed diverse thermotolerance levels among different strains, suggesting adaptation to specific environments. Younger silkworms exhibit more sensitivity to heat shocks, with thermotolerance increasing as they mature. Studies have highlighted the differential expression of heat shock proteins (HSPs) like 90 kDa and 84 kDa in early instars, and various HSPs in later stages, emphasizing their role in thermotolerance. In multivoltine strains, different sets of HSPs are expressed at various temperatures, indicating the importance of HSP concentration and distribution for acquiring thermotolerance. Recent research on Tasar silkworms has underscored the importance of HSPs in responding to thermal stress. The upregulation of *hsp21* and *hsp70* genes plays a critical role in cellular defense mechanisms during thermal stress. Insights from Kumar *et al.* (2011) have highlighted the role of *hsp21* in thermotolerance, while Vasudha *et al.* (2006) used proteomics to map HSP expression patterns across different strains and developmental stages, enhancing our understanding of thermal adaptation.

*In silico* approaches have become indispensable in studying thermal stress responses in Tasar silkworms.

Computational methods such as bioinformatics and molecular modeling are used to analyze and predict the behavior of key biomolecules involved in thermal stress response. These methods aid in identifying and characterizing HSPs like HSP21 and HSP70, which are crucial for cellular protection under high temperatures. *In silico* studies facilitate the exploration of protein interactions, structural changes, and expression patterns during thermal stress. Additionally, bioinformatics tools help identify regulatory elements within HSP gene sequences. Molecular dynamics simulations and *In silico* mutagenesis provide deeper insights into the structural dynamics and functional significance of these proteins under varying temperature conditions. These approaches offer a comprehensive framework for interpreting complex molecular responses, guiding experimental investigations, and developing strategies for breeding more thermotolerant silkworm strains.

This systematic review intended to investigate the vital role of molecular interventions in overcoming challenges in the Tasar silk industry, leading to the development of silkworm lines and technologies for various bio-active products.

## Improving Silk Yield in Silkworms: Molecular Insights and Breeding Strategies

To enhance silk yield in silkworms, understanding the molecular mechanisms involved in silk production is crucial, especially in strains with varying silk yields. Molecular studies, particularly using RNA sequencing (RNA-seq), can identify key genetic factors influencing silk yield. The development of markers associated with desirable traits, including silk yield, through RNA-seq holds promise for marker-assisted selection and breeding programs. Leveraging genetic resources from foreign strains, along with databases like SilkDB and KAIKObase, enhances our understanding and facilitates targeted breeding efforts (Table 1). Silkworm breeding plays a pivotal role in developing breeds tailored to specific regions or climates. While traditional breeding methods have been the norm, there's a growing need for new approaches like genetic engineering and biotechnological advancements. Molecular breeding, particularly Marker-Assisted Selection (MAS), proves to be a powerful tool in establishing specific traits within silkworm species. MAS accelerates the breeding process, providing accurate and precise genetic information, reducing the time needed for trait improvement, and enhancing selection efficiency.

**Table 1:** Genetic Databases for Silkworm Research

Database	Description
<b>SilkDB</b>	Provides essential genomic and transcriptomic information for <i>Bombyx mori</i> .
<b>KAIKObase</b>	A comprehensive database for silkworm genomics, offering valuable resources for researchers.
<b>SilkTransDB</b>	Focuses on transcriptomic data, aiding in the exploration of gene expression dynamics.
<b>SilkSatDb</b>	A database specifically dedicated to microsatellite markers in silkworm genomes.
<b>BmTEdb</b>	Offers information on transposable elements in the silkworm genome.

### Thermal stress and Heat Shock Proteins

The threshold temperature of mortality varies significantly among different lepidopteran species, reflecting their diverse thermotolerance levels. These variations highlight the importance of understanding species-specific thermal limits, particularly in the context of sericulture and the cultivation of heat-resistant silkworm strains like those of *Bombyx mori* (Table 2). Thermal tolerance is crucial for field-rearing, and polyvoltine silkworm strains generally exhibit better survivability than bivoltine strains. Studies on various strains reveal diverse thermotolerance levels, suggesting adaptation to specific environmental conditions. Bivoltine strains exhibit variable heat shock responses during different developmental stages, with younger silkworms being more sensitive to heat shocks. Thermotolerance increases as larval development progresses, indicating age-dependent resilience. Mortality thresholds vary among strains and species, emphasizing the need for strain-specific understanding. Investigating high thermo-resistance *in vivo* was proposed based on the observed cellular behavior. differential expression of HSPs, particularly 90 kDa and 84 kDa in early instars, and 84 kDa, 62 kDa, 60 kDa, 47 kDa, and 33 kDa in the fifth instar larvae, was reported at various heat shock temperatures (Vasudha *et al.*, 2006). In multivoltine strains (pure mysore, *C. nichi*), diverse sets of HSPs were expressed at different temperatures, with 90 kDa and 84 kDa being common (Joy and Gopinathan 1995; Evgen'ev *et al.*, 1987). The expression of HSPs varied across breeds, developmental stages, and temperatures, emphasizing the importance of concentration and distribution for thermotolerance acquisition (Joy and Gopinathan 1995; Kampinga 1993).

**Table 2:** Threshold temperature of Mortality in different lepidopteran

Species/Strain	Mortality Threshold (°C)
<i>Drosophila melanogaster</i>	40
<i>Bombyx mori</i> (various strains)	45-46
<i>Manduca sexta</i>	48
<i>Musca domestica</i>	46
<i>Lucilia cuprina</i>	48
<i>Locusta migratoria</i>	50

Notably, existing rearing practices, such as exposing young silkworm larvae to high temperatures, may contribute to stress-induced losses during summer, highlighting the importance of temperature management in optimizing cocoon production.

This in-depth exploration ventures into the intricate molecular responses of Tasar silkworms (*Antheraea mylitta*) when confronted with thermal stress, illuminating the pivotal role played by heat shock proteins (HSPs), specifically hsp21 and hsp70. As molecular chaperones, HSPs assume the crucial responsibility of safeguarding cellular proteins from denaturation, particularly under stress conditions such as exposure to elevated temperatures. The remarkable upregulation of hsp21 and hsp70 in response to thermal stress underscores their indispensable contribution to fortifying cellular defenses, positioning them as key players in the Tasar silkworm's adaptation to challenging environmental conditions. Building on the conserved function of HSPs observed across diverse organisms, the investigation extends its purview to explore genetic factors influencing thermal tolerance in Tasar silkworms. The seminal work by Kumar *et al.* in 2011 unravels the intricate molecular mechanisms governing the survival of silkworm strains, with a specific emphasis on the role of hsp21 in imparting thermotolerance. This genetic insight sheds light on the nuanced interplay between specific genes and the organism's ability to withstand thermal stress, offering a foundation for targeted breeding strategies aimed at enhancing thermotolerance in silkworm populations.

Employing a proteome approach, Vasudha *et al.* in 2006 contribute valuable insights into the multifaceted nature of the thermal stress response in Tasar silkworms. By unraveling distinct patterns of HSP expression across different silkworm strains and developmental stages, the study accentuates the complexity of the organism's response to thermal challenges. This comprehensive understanding aids in deciphering the intricate dynamics of thermal stress adaptation, emphasizing that the Tasar silkworm's

resilience is contingent on various factors, including strain and developmental stage. Genomic studies further enrich the narrative, characterizing specific HSPs such as the 90 kDa HSP and small heat shock proteins (sHSPs) in *Bombyx mori*, the domestic silkworm species closely related to the Tasar silkworm. This comparative genomics approach highlights the conserved nature of HSPs, emphasizing their fundamental role across silkworm species. Such genomic insights provide a broader context for understanding the molecular underpinnings of thermal stress responses, transcending species boundaries and contributing to our collective knowledge of HSP functionality.

Recent breakthroughs by Prabhu *et al.* in 2023 introduce a promising tool for advancing thermotolerance research – the DNA-based molecular marker, SCAR marker TT-PB1. This innovative marker, characterized by its specificity and reproducibility, offers a transformative avenue for analyzing the inheritance patterns of thermotolerance. The development of such molecular tools not only enhances our ability to select thermotolerant Tasar silkworm lines but also marks a crucial stride in bridging the gap between genetic understanding and practical applications in sericulture. Unraveling the differential expression patterns of HSPs in Tasar silkworms amid thermal stress not only elucidates their molecular resilience but also opens the gateway to broader applications. Beyond the realms of basic research, these findings hold implications for sericulture, biotechnology, and the sustainable utilization of Tasar silkworms. As we navigate through the intricate landscape of molecular intricacies, these collective insights promise to shape a comprehensive understanding of thermal stress tolerance in Tasar silkworms, influencing various scientific domains and practical applications in silk production.

Advancements in biotechnology, particularly genetic engineering, have revolutionized gene manipulation, allowing for precise alterations in organisms. Tasar silkworms (*Antheraea mylitta*) benefit from transgenic technology, which involves introducing foreign genes to confer specific traits. This approach addresses limitations in conventional breeding methods by enhancing silk quality, tensile strength, and the production of value-added proteins. Genetic modifications, achieved through recombinant DNA technology, enable the introduction of genes encoding beneficial proteins, expanding the scope beyond silk production. Transgenic technology has also been explored to bolster disease resistance, introducing genes associated with immune responses

or antimicrobial peptides. Notably, research in *Bombyx mori* has demonstrated the transformative potential of germline transformation, allowing for the insertion of target genes into silkworm chromosomes. This breakthrough has paved the way for the development of transgenic silkworms acting as bioreactors for producing recombinant proteins and superior silk. Ongoing efforts extend to applications in drug development, including cancer drugs and monoclonal antibody drugs. While promising, the introduction of transgenes necessitates careful consideration of environmental risks and biodiversity impact. In addressing challenges related to the pupal diapause phenomenon in Tasar silkworms, a study identified diapause-specific expressed sequence tags (ESTs) associated with various genes, shedding light on the molecular aspects of diapause induction, maintenance, and termination. This molecular understanding provides insights for the effective management and utilization of Tasar silkworms in silk production, particularly in addressing issues related to pupal diapause and enhancing their economic significance.

The Daba bivoltine ecorace of the tropical tasar silkworm *Antheraea mylitta Drury* exhibits facultative pupal diapause, leading to erratic and unsynchronized adult emergence during its extended pupal diapause period. This phenomenon results in substantial losses of seed stock, ranging from 10 to 30%. To address these challenges and enhance understanding of diapause induction, maintenance, and termination in this economically significant insect, the study identified diapause-specific expressed sequence tags (ESTs) through PCR clones of various genes, including Hsp70, Hsp23, hexamerins, and PCNA. Hexamerins' ESTs were detected only in pupae aged 65 and 165 days, while Hsp70 sequences exhibited upregulation during early, middle, and late stages of the diapause period. Hsp23 presence was observed during the preparatory phase of diapause (IV instar) and early to mid-aged pupae (D0 to D75), continuing through the late diapause stage (D135 to D165). Hsp22 ESTs were identified during the preparatory phase, throughout diapause, and even after diapause termination. Hsps90 showed upregulation during the preparatory stage and the middle to late diapause period. PCNA ESTs exhibited downregulation throughout diapause, with upregulation at diapause termination. Another set of Hsp70 primers revealed intermittent upregulation throughout diapause, and Hsps90 exhibited upregulation during the middle and late diapause stages. Notably, ESTs disappeared in pupae older than 165 days, coinciding with diapause termination, supported by the upregulation of PCNA ESTs. Pupae aged 195 days and older can be strategically exploited

for low-temperature treatment, delaying moth emergence in adverse summer conditions to align with the cropping schedule. This comprehensive molecular understanding offers insights for effective management and utilization of the Daba bivoltine ecorace in tasar silk production.

### Molecular Marker in Sericulture

In recent years, significant strides have been made in silkworm research, particularly in understanding genetic diversity through the utilization of molecular markers (Table 3). *Antheraea Mylitta D.*, a lepidopteran insect belonging to the Saturniidae family, is known for producing tasar silk of commercial importance. This species exhibits distinct ecological races distributed across various geographical regions in India, each showcasing variations in phenotypic traits such as fecundity, voltinism, cocoon weight, silk ratio, and host plant preference. The diminishing number of ecoraces and challenges in their identification have prompted the development of molecular markers for deciphering genetic closeness and identifying wild silkworm ecoraces (Saha and Kundu, 2006). Notable markers like Genomic-SSR, Sequence-Related Amplified Polymorphism (SRAP), Internal Transcribed Spacer (ITS), Start Codon Targeted Polymorphism (SCoT), Cleaved Amplified Polymorphic Sequences (CAPS) and Direct Amplification of Minisatellite DNA (DAMD) have been employed (Krishnan RR *et al.*, 2013; Srivastava *et al.*, 2004; Wei-Guo *et al.*, 2006; Rohela *et al.*, 2020; Varora *et al.*, 2017; Bhattacharya & Ranade, 2001).

Ashok Kumar *et al.* (2011) conducted a study using isozyme analysis to assess genetic diversity and thermotolerance among silkworm races. The results

highlighted the potential of isozyme analysis in grouping diverse genotypes based on origin and thermotolerance, offering insights into the adaptive strategies of different genotypes within a population. The study emphasized the utility of isozyme profiles in providing reliable estimates of genetic diversity for the strategic development of improved silkworm hybrids. Additionally, genomic information has been harnessed for SNP markers to identify genetic differentiation in *Bombyx mori*, contributing to the exploration of domestication events (Xia *et al.*, 2009). The use of SNP DNA markers has been extended to study cocoon traits in mulberry silkworms, offering insights into specific traits linked to SNP markers (Sreekumar *et al.*, 2011). Furthermore, studies utilizing RAPD profiles have demonstrated the high sensitivity and efficiency of this technique in detecting differences and polymorphism among silkworm strains. The analysis of genetic diversity and phylogenetic relationships among silkworm species and races using RAPD markers has revealed valuable insights into their genetic makeup (Singh *et al.*, 2011; Kartik Neog *et al.*, 2010; Velu *et al.*, 2008). The pioneering work in silkworm genome sequencing by Mita *et al.* (2003) and subsequent comprehensive shotgun sequencing by Mita *et al.* (2004) and Xia *et al.* (2004) has laid the foundation for understanding the genomic landscape of *Bombyx mori*. This genomic information has been used to identify SNP markers, enhancing the study of genetic diversity and structure in silkworm populations (Yamamoto *et al.*, 2008). The integration of traditional breeding methods with genomic insights offers promising avenues for further improvements in silk characteristics and mulberry plant traits, contributing to the sustainable development of sericulture.

**Table 3:** Molecular Markers Used in Tasar Silkworm Research

Researchers and Year	Molecular Markers Used	Focus of Study	Key Findings and Significance
Chatterjee <i>et al.</i> (2004)	RAPD Marker	Polymorphism between Raily Ecorace Populations	Identified significant DNA polymorphism in Raily ecoraces
Kar <i>et al.</i> (2005)	ISSR Markers	Genetic Diversity in Semi-Domesticated Daba	Notable variations within and between Daba ecorace groups
Vijayan <i>et al.</i> (2005)	ISSR and RAPD Markers	Genetic Variation between Raily, Daba, and Modal Ecoraces	Limited genetic variation among ecoraces, establishing stability
Ghosh <i>et al.</i> (2005)	Retrotransposons	Tasar Silkworm Pao-Like Long Terminal Repeats	Contributed to molecular characterization of retrotransposons
Saha and Kundu (2006)	SCAR and RAPD Markers	Molecular Identification of Ecoraces	Discriminated eight out of ten ecoraces using RAPD-selected bands
Mahendran <i>et al.</i> (2006)	RFLP Technique	Genetic Variation in Nine Ecoraces	Linked genetic variations with phenotypic and geographical differences

Mahendran <i>et al.</i> (2006)	Cytochrome Oxidase and 16S rRNA	Molecular Phylogeny	Explored silkworm molecular phylogeny using genomic information
Velu <i>et al.</i> (2008)	ISSR Primers	Genetic Links in Mutant Silkworm Stocks	Identified sub-clusters, emphasized significant variation
Saha <i>et al.</i> (2008)	RAPD Markers	Genetic Differentiation in Commercial Ecoraces	Provided insights into genetic variability for breeding strategies
Xia <i>et al.</i> (2009)	SNP Markers	Genetic Differentiation in Domesticated and Wild Silkworm Strains	Explored domestication events and genetic differentiation
Chakraborty <i>et al.</i> (2015)	Microsatellite Markers	Population Structure of Indian Tasar Silkworm Population	Enhanced resolution in identifying genetic variation and population structure
Renuka and Shamitha (2016)	SSR and ISSR Markers	Phylogenetic Analysis and Genetic Variation in Tasar Silkworm Ecoraces	Contributed to understanding genetic structure and evolutionary links
Niranjan <i>et al.</i> (2018)	RAPD-SCAR Markers	Selection of Daba BV High Shell Weight Line	Developed SCAR markers for selecting high silk yield lines
Prabhu <i>et al.</i> (2023)	RAPD-SCAR Markers	Selection of Thermo-tolerant Line of Tasar Silkworms	Developed SCAR markers for selecting thermo-tolerant lines

Disease resistance in silkworms is a critical aspect of sericulture, ensuring the health and productivity of silkworm populations. Various techniques and strategies have been employed to enhance disease resistance in silkworms, addressing challenges posed by infectious agents (Table 4).

**Table 4:** Techniques for Disease Resistance in Silkworms

Technique	Application	References
RNAi (RNA interference)	Enhancing resistance against viral, bacterial, and parasitic infections	J. Xu <i>et al.</i> (2016); Guan <i>et al.</i> (2018)
siRNA (short interfering RNA)	Targeting genes associated with susceptibility to pathogens	Guan <i>et al.</i> (2018)
Hairpin RNA sequences	Designing constructs for RNAi to suppress specific genes	Guan <i>et al.</i> (2018)
Microinjection and feeding	Delivery methods for introducing RNAi molecules into silkworms	Guan <i>et al.</i> (2018)
qPCR (quantitative PCR)	Molecular techniques to confirm downregulation of specific genes in RNAi studies	Guan <i>et al.</i> (2018)

### Future approaches in tasar silkworm

In the study of thermal stress responses in Tasar silkworms (*Antheraea mylitta*), *In silico* approaches play a pivotal role in providing valuable insights into the molecular mechanisms governing heat tolerance. Computational methods, such as bioinformatics and molecular modeling, are employed to analyze and predict the behavior of key biomolecules involved in thermal stress response. These approaches aid in the identification and characterization of heat shock proteins (HSPs), such as HSP21 and HSP70, crucial for cellular protection during elevated temperatures. *In silico* studies facilitate the exploration of protein-protein interactions, structural changes, and expression patterns under thermal stress conditions. Additionally,

bioinformatics tools contribute to the identification of potential regulatory elements within the genomic sequences of HSP genes. Molecular dynamics simulations and *In silico* mutagenesis provide a deeper understanding of the structural dynamics and functional significance of these proteins under varying temperature conditions. Overall, *In silico* approaches enhance the efficiency of thermal stress studies in Tasar silkworms by offering a computational framework to interpret complex molecular responses, guiding further experimental investigations and contributing to the development of strategies for breeding more thermotolerant silkworm strains (Table 5).



**Table 5:** *In-silico* approaches in tasar silkworm

Approach	Application	Benefits	Impact
Whole-Genome Shotgun Sequencing (WGS)	Identifying genes associated with silk production, exploring genetic diversity, and understanding evolutionary relationships in Tasar silkworms.	Facilitates functional annotation, pathway analysis, and molecular marker development.	Improves sericulture practices, guiding breeding strategies, and contributes to overall silk production.
Next-Generation Sequencing (NGS)	Unraveling Tasar silk genome intricacies, identifying silk production-related genes, and understanding genetic variations.	High-throughput, cost-effective, and rapid sequencing capabilities.	Accelerates advancements in sericulture, guides research initiatives, and enhances silk production.
RNA Sequencing (RNA-seq)	Examining gene expression changes in response to environmental factors, identifying novel transcripts and variations in the Tasar silk genome.	Offers insights into functional elements, biological pathways, and gene expression dynamics.	Contributes to targeted breeding programs, facilitates marker-assisted selection, and improves silk yield traits.
CRISPR-Cas9 Technology	Investigating gene function in <i>Antheraea mylitta</i> , targeting specific genes within the Tasar silkworm genome.	Enables precise gene editing, leading to potential modifications in silk-related genes.	Revolutionize the silk industry by enhancing production and modifying silk properties.
<i>In silico</i> Approaches (Bioinformatics)	Leveraging computational tools to analyze and interpret genomic data, predict gene functions, and identify regulatory elements.	Provides efficient and cost-effective means for data analysis, gene prediction, and functional annotation.	Enhances understanding of molecular mechanisms, aids in gene discovery, and guides molecular breeding efforts.
<i>In silico</i> Molecular Docking Studies	Predicting interactions between molecules, aiding in the design of potential silk-enhancing compounds.	Screens virtual compounds for their binding affinity to silk-related proteins.	Accelerates drug discovery for improving silk characteristics, potentially impacting silk quality and properties.

These approaches are vital for overcoming challenges and advancing the tasar silk industry by developing innovative silkworm strains and technologies for various bio-active products.

### Conclusion

The advancements in molecular interventions have significantly transformed sericulture, particularly in the context of the tropical Tasar silkworm, *Antheraea mylitta*. By delving into the molecular mechanisms of silk production, thermal tolerance, and disease resistance, researchers have paved the way for substantial improvements in silk yield and overall silkworm health.

The enhancement of silk yield hinges on a deep understanding of the genetic factors influencing silk production. RNA sequencing (RNA-seq) has been instrumental in identifying these key genetic elements. The development of markers associated with desirable traits, such as silk yield, through RNA-seq is promising for marker-assisted selection and targeted breeding programs. Utilizing genetic resources from foreign strains and comprehensive databases like SilkDB and

KAIKObase has significantly bolstered breeding efforts. Marker-Assisted Selection (MAS) emerges as a powerful tool, accelerating the breeding process by providing precise genetic information, thereby reducing the time required for trait improvement and enhancing selection efficiency. The integration of traditional breeding methods with modern genetic engineering and biotechnological advancements further enhances the potential for developing high-yielding silkworm strains.

Thermal tolerance is a critical aspect of silkworm rearing, with polyvoltine strains generally exhibiting better survivability than bivoltine strains. Studies have highlighted the diverse thermotolerance levels among different strains, emphasizing the need for strain-specific understanding. The differential expression of heat shock proteins (HSPs) such as 90 kDa and 84 kDa in early instars, and various HSPs in later stages, plays a crucial role in thermotolerance. Research on Tasar silkworms has underscored the significance of HSPs like hsp21 and hsp70 in responding to thermal stress. The upregulation of these genes highlights their essential role in cellular defense mechanisms. Insights

from proteomic studies further elucidate the complex dynamics of HSP expression across different strains and developmental stages, contributing to a comprehensive understanding of thermal adaptation.

*In silico* approaches have become indispensable in studying thermal stress responses and other molecular mechanisms in Tasar silkworms. Computational methods such as bioinformatics and molecular modeling are employed to analyze and predict the behavior of key biomolecules involved in thermal stress response. These methods aid in identifying and characterizing HSPs, exploring protein interactions, structural changes, and expression patterns under stress conditions. Bioinformatics tools also contribute to identifying regulatory elements within HSP gene sequences. Molecular dynamics simulations and *In silico* mutagenesis provide deeper insights into the structural dynamics and functional significance of these proteins under varying temperature conditions. These approaches offer a comprehensive framework for interpreting complex molecular responses, guiding experimental investigations, and developing strategies for breeding more thermotolerant silkworm strains.

The integration of molecular interventions in sericulture, particularly for Tasar silkworms, holds immense potential for overcoming existing challenges and advancing the industry. The development of innovative silkworm strains and technologies for various bio-active products not only enhances silk production but also contributes to the sustainable development of sericulture. As research continues to unravel the intricate molecular mechanisms underlying silk production, thermal tolerance, and disease resistance, the future of Tasar silkworms in sericulture looks promising, with far-reaching implications for biotechnology and sustainable agriculture.

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