



## Molecular Biomarkers for Silkworm Stress Detection: A New Tool for Sustainable Sericulture

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Sericulture is a climate-sensitive agro-industry in which the productivity and health of the silkworm (*Bombyx mori*) are greatly influenced by environmental and biological stresses. Conventional methods of stress detection rely mainly on visible symptoms, which often appear only after significant physiological damage has occurred. In this context, molecular biomarkers have emerged as reliable tools for early stress detection. Molecular biomarkers such as heat shock proteins, antioxidant enzymes, immune-related genes, microRNAs, and metabolic indicators provide valuable insights into the physiological status of silkworms under stress conditions. These biomarkers can be identified using modern molecular techniques such as PCR, transcriptomics, proteomics, and metabolomics. Early detection of stress through these indicators can help in timely disease management, improvement of silkworm breeds, and maintenance of silk quality. Furthermore, molecular biomarkers play an important role in developing stress-resilient silkworm varieties suited to changing climatic conditions. Although challenges such as cost and technical expertise limit their widespread adoption, advances in biotechnology may soon make these tools more accessible for practical sericulture. Thus, the application of molecular biomarkers represents a promising approach toward sustainable and precision sericulture.

**Keywords:** Sericulture, *Bombyx mori*, molecular biomarkers, stress detection, heat shock proteins, gene expression, silk productivity.

### Introduction

Sericulture is an important agro-based rural industry that supports millions of farmers worldwide, particularly in countries like India, China, and Japan. The success of sericulture largely depends on the health and productivity of the silkworm *Bombyx mori*. However, silkworms are highly sensitive to environmental fluctuations such as temperature, humidity, nutrition, pesticides, and pathogen attack. Even slight stress can reduce cocoon quality and silk yield, causing significant economic losses. Traditionally, stress in silkworms has been detected through visible symptoms such as reduced feeding, sluggish movement, uneven growth, or disease appearance. However, these symptoms often appear only after damage has already occurred. Modern molecular biology now offers advanced tools to detect stress at an early stage through molecular biomarkers. Molecular biomarkers are measurable biological molecules such as genes, proteins, or metabolites that indicate physiological stress. These biomarkers help in early diagnosis, improving disease management and increasing silk productivity. This article explains the importance, types, and applications of molecular biomarkers in detecting stress in silkworms.

### Stress in Silkworms

Stress in silkworms refers to any environmental or biological factor that disturbs normal physiological processes. These stress factors can be broadly classified into:

**Abiotic stress**

Abiotic stress includes environmental factors such as:

- High temperature
- Low temperature
- Humidity fluctuations
- Poor nutrition
- Chemical exposure
- Pesticide residues, among these, heat stress is one of the most serious problems affecting silkworm rearing, especially under tropical conditions.

**Biotic stress**

Biotic stress includes biological factors such as:

- Bacterial diseases
- Viral infections
- Fungal diseases
- Protozoan infections like pebrine
- Parasitic infestations, these stressors affect metabolism, immunity, and silk gland development, ultimately affecting silk production.

**What are Molecular Biomarkers**

Molecular biomarkers are biological indicators found at the molecular level that reflect physiological changes. These may include

- DNA markers
- RNA transcripts
- Proteins
- Enzymes
- Metabolites

When silkworms are exposed to stress, specific genes become activated or suppressed. These changes can be detected using molecular techniques such as

- PCR (Polymerase Chain Reaction)
- qRT-PCR
- Transcriptomics
- Proteomics
- Metabolomics, these tools help detect stress before physical symptoms appear, enabling preventive management.

**Types of Molecular Biomarkers in Silkworm Stress Detection****Heat shock proteins (HSPs)**

Heat shock proteins are among the most important molecular biomarkers used in stress detection. These proteins act as molecular chaperones that protect cells from damage caused by stress conditions. When silkworms experience heat stress, HSP genes such as **HSP70**, **HSP90**, and small heat shock proteins become highly expressed. These proteins help maintain protein structure and prevent cellular damage. Studies show that heat shock proteins are significantly upregulated during thermal stress and play an important role in silkworm thermotolerance. Because of their rapid response, HSPs are considered reliable early stress indicators.

**Antioxidant enzymes**

Stress conditions often cause oxidative damage in silkworm tissues. This leads to the production of reactive oxygen species (ROS), which damage cells. To counter this, silkworms produce antioxidant enzymes such as

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Peroxidase (POD)
- Glutathione S-transferase (GST), changes in the levels of these enzymes can serve as biomarkers for oxidative stress detection.

**Immune-related genes**

Silkworms possess innate immunity that responds to pathogens through antimicrobial peptides and immune proteins. Important immune biomarkers include:

- Cecropins
- Defensins
- Lysozyme
- Attacin genes, upregulation of these genes indicates pathogen infection or immune stress. Molecular monitoring of these genes helps detect disease outbreaks at early stages.

**Metabolic biomarkers**

Stress alters metabolic pathways in silkworms. Changes in metabolites such as amino acids, sugars, and lipids can indicate stress conditions.

Metabolomics studies have shown that stress can affect:

- Energy metabolism
- Protein synthesis
- Silk protein formation
- Hormonal balance, such metabolic changes can serve as indicators of physiological disturbances.

**MicroRNA biomarkers**

MicroRNAs (miRNAs) are small regulatory RNA molecules that control gene expression. Recent research suggests that miRNAs play important roles in:

- Stress response
- Development
- Immunity
- Metabolism

Changes in miRNA expression can therefore be used as sensitive biomarkers of stress response.

**Molecular Techniques Used in Stress Biomarker Detection**

Modern molecular biology provides several techniques for identifying stress biomarkers.

**Polymerase Chain Reaction (PCR)**

PCR is widely used for detecting gene expression changes during stress. Quantitative PCR helps measure gene activity levels under stress conditions.

**Transcriptomics**

Transcriptomics studies all RNA molecules expressed in a cell. This helps identify stress-responsive genes.

Transcriptomic studies have shown that thermal stress activates multiple stress response pathways, including protein processing and longevity regulation pathways.

**Proteomics**

Proteomics analyzes protein expression changes under stress conditions. This helps identify stress-responsive proteins affecting silk quality.

**Metabolomics**

Metabolomics studies metabolic changes occurring due to stress. This provides a comprehensive view of physiological changes.

**Importance of Molecular Biomarkers in Sericulture****Early stress detection**

Molecular biomarkers allow detection of stress before visible symptoms appear. This helps farmers take preventive measures.

**Improving disease management**

Biomarkers help identify disease infection at early stages, improving control strategies and reducing crop losses.

**Breed improvement programs**

Molecular biomarkers help identify stress-tolerant silkworm breeds. These markers are useful in breeding programs aimed at improving resilience.

### **Climate change adaptation**

Climate change is increasing temperature fluctuations, making stress detection more important. Biomarkers can help identify climate-resilient silkworm varieties.

### **Precision sericulture**

Molecular biomarkers are contributing to precision sericulture, where scientific monitoring improves productivity.

### **Stress Biomarkers Related to Silk Production**

Stress directly affects silk gland development and silk protein synthesis. Molecular studies show that stress alters fibroin and sericin gene expression as a result

- Cocoon weight decreases
- Filament length reduces
- Silk strength declines, monitoring molecular biomarkers helps protect silk quality.

### **Role of Heat Shock Proteins as Ideal Biomarkers**

Among all molecular biomarkers, heat shock proteins are considered the most reliable. These proteins respond quickly to stress and can be easily detected. Research indicates that genes such as Hsp90 and Hsp19.9 play significant roles in heat tolerance and survival under stress conditions. Their advantages include

- Rapid response to stress
- High sensitivity
- Easy detection
- Strong correlation with survival

Because of these features, HSPs are widely used as stress biomarkers in sericulture research.

### **Future Prospects**

The future of molecular biomarker research in sericulture is very promising. Emerging technologies such as:

- Next generation sequencing
- CRISPR gene editing
- Artificial intelligence
- Biosensors may revolutionize stress detection.

Portable diagnostic kits based on molecular biomarkers may soon help farmers detect stress directly in rearing houses.

Integration of omics technologies may also help develop:

- Stress-resistant silkworm breeds
- Disease-resistant strains
- Climate-adapted varieties

### **Challenges in Using Molecular Biomarkers**

Despite their advantages, some challenges remain:

- High cost of molecular tools
- Need for technical expertise
- Limited field-level application
- Lack of awareness among farmers. However, as technology becomes cheaper, these tools may become more accessible.

### **Conclusion**

Molecular biomarkers represent a powerful new approach for detecting stress in silkworms. Unlike traditional observation methods, molecular indicators provide early warning signals that help prevent losses. Heat shock proteins, antioxidant enzymes, immune genes, and metabolic markers are important indicators of stress response. The use of molecular biomarkers can significantly improve disease management, breed improvement, and climate resilience in sericulture. As molecular tools become more affordable and accessible, their adoption in sericulture will increase. In the future, combining molecular biology with

traditional sericulture practices may lead to more sustainable and profitable silk production. Thus, molecular biomarker research represents an important step toward modernizing sericulture.

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