

INTEGRATING MOLECULAR BIOLOGY, CLIMATE RESILIENCE AND SUSTAINABLE MULBERRY PRODUCTION TO IMPROVE SILKWORM PERFORMANCE AND SILK PRODUCTIVITY UNDER CHANGING ENVIRONMENTAL CONDITIONS

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ABSTRACT

Sericulture represents an important agro-based livelihood system that connects mulberry cultivation, insect physiology, silk production, and rural economic development. However, rising temperatures, irregular rainfall, drought, humidity fluctuations, and increasing environmental variability are creating new challenges for mulberry productivity, silkworm development, disease resistance, and silk quality. Addressing these challenges requires an integrated approach that combines molecular biology, climate-resilient crop management, silkworm genetics, and sustainable agricultural practices. This review examines the molecular and physiological mechanisms governing silkworm responses to environmental stress, with particular emphasis on gene expression regulation, heat-shock proteins, antioxidant defence, immune signalling, hormonal regulation, and molecular pathways associated with silk-gland development and silk-protein synthesis. It also evaluates how mulberry genotype selection, soil management, irrigation efficiency, nutrient recycling, and biological approaches to pest and disease management can contribute to a more resilient sericulture production system. Emerging technologies, including transcriptomics, genomics, epigenetics, metabolomics, and integrated multi-omics, offer opportunities to identify stress-responsive genes, molecular markers, and candidate targets for breeding climate-resilient silkworm strains and high-quality mulberry cultivars. The review further considers the potential of molecular-assisted breeding and environmentally responsible production strategies to improve the stability of cocoon yield, silk filament characteristics, and resource-use efficiency. Important research gaps include the limited integration of molecular responses with long-term field performance, insufficient multi-location validation of stress-tolerant genotypes, and the need to evaluate the interaction between host-plant quality, silkworm microbiota, and environmental stress. A systems-level framework is proposed to connect molecular discoveries with practical sericulture management, supporting climate adaptation, sustainable silk production, and livelihood resilience.

KEYWORDS: Sericulture; *Bombyx mori*; *Morus alba*; molecular biology; climate resilience; heat-shock proteins; transcriptomics; silk-gland development; sustainable mulberry cultivation; silk productivity.

1. INTRODUCTION

Sericulture is a biologically interconnected agricultural production system in which the quality of the host plant, the developmental physiology of the silkworm, environmental conditions, and farm management collectively determine the quantity and quality of silk. Mulberry silkworms, particularly *Bombyx mori*, depend on mulberry leaves as their principal food source, making the nutritional and physiological condition of the host plant a fundamental determinant of larval growth, cocoon formation, and silk production. The performance of this system is influenced by several interacting factors, including mulberry genotype, leaf moisture and nutrient composition, larval density, temperature, relative humidity, disease pressure, and the management practices adopted during rearing. Consequently, improving sericultural productivity requires approaches that consider both plant and insect biology rather than treating mulberry cultivation and silkworm rearing as independent activities.

Climate change introduces additional complexity into this relationship. Elevated temperatures can disturb silkworm development, increase metabolic demands, alter feeding behaviour, and affect the synthesis and organization of silk proteins. Similarly, drought and irregular precipitation can reduce mulberry growth, alter leaf nutritional composition, and constrain the availability of suitable foliage during critical larval stages. High humidity, inadequate ventilation, and unstable rearing conditions may also influence pathogen transmission and disease development. These stresses do not necessarily act independently; their combined effects may be more damaging than those produced by individual environmental factors. The magnitude of these effects varies with silkworm genetic background, developmental stage, exposure duration, host-plant condition, and the capacity of farmers to regulate the rearing environment.

Molecular biology offers a means of understanding how these environmental pressures affect sericulture at the cellular and genetic levels. Silkworm responses to heat, oxidative stress, nutritional limitation, and infection

involve coordinated changes in gene expression, protein stability, cellular signalling, metabolism, and immune function. Heat-shock proteins, antioxidant enzymes, immune-related molecules, and regulators of endocrine development are among the components that may influence stress tolerance and developmental stability. At the same time, genes involved in silk-gland differentiation and the synthesis of fibroin and sericin determine important aspects of silk formation. Investigating the relationships among these molecular processes can help explain why particular silkworm strains maintain better growth or cocoon characteristics under challenging environmental conditions.

Parallel advances in mulberry biotechnology have expanded the opportunities to improve host-plant performance. Genetic diversity within *Morus* species can provide useful traits related to biomass production, leaf quality, drought response, and adaptation to different growing conditions. Molecular markers, genomic resources, transcriptome analysis, and functional studies can support the identification of genes associated with these traits. Nevertheless, the agronomic value of a molecularly identified trait depends on its expression under practical cultivation conditions and its contribution to silkworm performance. A mulberry genotype with improved drought tolerance, for example, will be especially valuable to sericulture when it maintains suitable leaf biomass and nutritional quality during water limitation.

The central challenge is therefore to connect molecular mechanisms with measurable production outcomes. Molecular stress responses must be interpreted alongside larval survival, developmental duration, cocoon weight, shell ratio, filament length, silk composition, and the resource requirements of mulberry cultivation and silkworm rearing. Likewise, sustainability must be evaluated beyond yield alone by considering water and nutrient efficiency, soil health, pesticide dependence, energy consumption, waste management, and the economic feasibility of recommended interventions. This review integrates these dimensions to examine how molecular biology, climate-resilient mulberry production, and sustainable management can jointly strengthen silkworm performance and silk productivity under changing environmental conditions.

2. Climate Change and Its Implications for Sericulture

2.1 Temperature stress and silkworm development

Temperature is a major environmental regulator of silkworm physiology, influencing larval feeding, moulting, metabolic activity, immune competence, silk-gland development, and cocoon formation. Although silkworm strains differ in their thermal tolerance, exposure to temperatures outside their suitable rearing range can disrupt developmental processes and reduce production stability. The effects depend not only on the maximum temperature reached but also on the duration of exposure, the developmental stage, humidity, and the availability of adequate nutrition.

Experimental evidence demonstrates that temperature-related changes extend to the molecular regulation of silkworm development. A transcriptomic investigation comparing *B. mori* larvae maintained at 26 °C and 34 °C during the fifth instar identified 3,115 differentially expressed transcripts. The affected genes included those associated with immune responses, transcriptional regulation, silk biosynthesis, and heat-shock responses. Larvae exposed to 34 °C also exhibited reduced body weight and lower cocoon production performance, illustrating how thermal stress can connect molecular disruption with commercially important traits.

The relationship between temperature and productivity is not necessarily linear. Mild, short-duration thermal conditioning may activate protective responses, whereas prolonged or severe heat exposure can exceed the insect's capacity for physiological adjustment. Recent research has shown that heat hardening can improve subsequent survival under severe heat stress and that particular heat-shock proteins contribute differently to this response. These findings indicate that thermal adaptation is regulated by specific molecular components rather than by a uniform increase in all stress-response proteins.

From a production perspective, the critical question is whether observed thermotolerance translates into sustained larval growth, successful cocooning, and acceptable silk quality under realistic rearing conditions. Survival alone is insufficient as a selection criterion because individuals may survive stress while exhibiting delayed development, reduced feeding, lower cocoon weight, or impaired silk conversion. Future assessments should therefore combine molecular indicators with multiple economic traits across different larval stages.

2.2 Drought, irregular rainfall, and mulberry leaf quality

Mulberry is the nutritional foundation of conventional sericulture, and its performance depends on soil moisture, nutrient availability, temperature, light, and genotype. Drought can limit leaf expansion, reduce biomass accumulation, alter photosynthetic activity, and change the concentration and balance of nutrients available to feeding larvae. These changes may affect silkworm growth even when the rearing environment itself is adequately controlled.

The consequences of drought extend beyond a reduction in leaf quantity. Changes in leaf water content, soluble sugars, amino acids, structural carbohydrates, and secondary metabolites may influence palatability, digestibility, and nutrient assimilation. However, the direction and magnitude of these changes depend on the mulberry genotype, stress intensity, developmental stage of the plant, and the timing of leaf sampling. Consequently, drought-resistant mulberry should not be defined exclusively by its capacity to survive water deficit; it should also maintain sufficient leaf yield and nutritional suitability for silkworm feeding.

Combined heat and drought are particularly important because the two stresses can interact at physiological and molecular levels. An integrated transcriptomic and proteomic study of *Morus alba* examined responses to combined drought and heat stress and identified candidate regulatory components, including HSP21, HSP70T-2,

PPH, BAG6, MybSt1, and GT2. The study provides a molecular basis for investigating stress-response networks in mulberry, although the contribution of each candidate to field-level leaf yield and silkworm productivity requires further validation.

Irregular rainfall may also create alternating periods of water shortage and excessive soil moisture. These conditions can complicate irrigation scheduling, nutrient uptake, root health, and disease management. Sustainable mulberry cultivation therefore requires soil-moisture monitoring, locally appropriate irrigation, maintenance of soil organic matter, and evaluation of cultivars under the water regimes that farmers are likely to experience.

2.3 Humidity, disease pressure, and environmental interactions

Relative humidity influences water loss, larval physiology, leaf moisture, and the microenvironment within silkworm rearing facilities. Its effects interact with temperature, ventilation, stocking density, sanitation, and pathogen exposure. High temperature combined with inadequate ventilation may create conditions that compromise larval performance, while unsuitable moisture conditions can influence the persistence or transmission of certain pathogens.

Climate-related disease risk should not be interpreted as a universal increase in all silkworm infections. Different pathogens respond differently to temperature and moisture, and outbreaks depend on the interaction among pathogen biology, host susceptibility, rearing practices, and environmental conditions. Molecular investigations can help distinguish whether a decline in larval performance is associated primarily with thermal injury, immune suppression, infection, or nutritional stress.

A more informative research design would monitor temperature, humidity, leaf quality, pathogen load, and host gene expression simultaneously. Such integrated measurements would help identify the conditions under which environmental stress increases disease susceptibility and determine whether improved rearing management can reduce the need for chemical interventions.

3. Molecular Mechanisms Underlying Silkworm Stress Tolerance

3.1 Heat-shock proteins and cellular protection

Heat-shock proteins (HSPs) are molecular chaperones involved in protein folding, stabilization, and cellular recovery under stress. Their importance in silkworm thermal biology has been documented across multiple HSP families, particularly HSP70 and HSP90. These proteins may help protect cellular functions when elevated temperature disturbs protein structure and intracellular homeostasis. However, HSP expression varies among tissues, developmental stages, sexes, and genetic backgrounds, making it necessary to interpret expression patterns within their biological context.

The HSP70 family is of particular interest because its members participate in protein quality control and responses to multiple abiotic stressors. Nevertheless, the presence of an HSP70 gene or its increased expression does not automatically establish that a silkworm strain is more heat tolerant. Some genes may respond strongly to stress without providing sufficient protection, while others may be essential at particular developmental stages or under specific exposure conditions. Molecular marker development should therefore be supported by experiments that establish relationships between gene variation or expression and independently measured performance.

Recent functional evidence strengthens this distinction. In a 2025 study, heat hardening increased survival during subsequent severe heat exposure, and RNA interference experiments identified important roles for HSP90 and HSP19.9 in thermal adaptation. The absence of an equivalent survival effect following the knockdown of other tested HSPs demonstrates that members of the same general protein family can have different functional contributions.

For sericulture improvement, these findings support three complementary research directions. First, researchers should identify genetic variation in HSP-related genes among commercial and indigenous silkworm strains. Second, expression patterns should be measured under controlled and fluctuating temperatures to distinguish constitutive tolerance from stress-induced acclimation. Third, candidate genes should be evaluated against larval survival, developmental duration, cocoon weight, shell ratio, and filament characteristics. This approach would prevent the selection of molecular markers that indicate stress exposure but do not reliably predict productive performance.

3.2 Oxidative stress and antioxidant defence

Environmental stress can disturb cellular redox balance and increase the formation of reactive oxygen species. At controlled levels, reactive oxygen species participate in signalling; when their production exceeds cellular antioxidant capacity, oxidative damage may affect proteins, lipids, nucleic acids, and membrane integrity. Silkworm responses to thermal and nutritional stress therefore need to be examined in relation to both oxidative damage and the mechanisms that maintain redox homeostasis.

Antioxidant defence involves interacting enzymatic and non-enzymatic systems. Superoxide dismutase contributes to the conversion of superoxide radicals into hydrogen peroxide, while catalase and several peroxidases help remove hydrogen peroxide. Glutathione-dependent systems, redox-regulating proteins, and small antioxidant molecules provide additional protection. Their activities should be interpreted alongside measurements of oxidative damage and physiological performance rather than used individually as evidence of tolerance.

A molecular study of stress resilience could measure antioxidant enzyme activities, expression of relevant genes, glutathione status, lipid peroxidation, and protein oxidation in different silkworm tissues. Combining these

measurements with survival and cocoon traits would help determine whether antioxidant responses represent effective protection or merely reflect a stronger stress burden.

The same principle applies to mulberry plants. Drought and heat can alter photosynthetic function and cellular redox balance, but effective stress tolerance requires coordination among antioxidant defence, water regulation, carbon assimilation, and growth. Identifying genotypes that maintain both physiological stability and useful leaf production is more valuable than selecting plants solely for a strong antioxidant response.

3.3 Immune signalling and disease resistance

Silkworm immune function is another important component of climate resilience. Environmental stress can influence cellular integrity, haemocyte activity, antimicrobial responses, and the regulation of immune-related genes. These responses are relevant to bacterial, fungal, viral, and microsporidian infections, although the mechanisms and outcomes differ according to the pathogen involved.

Temperature-associated transcriptomic changes in *B. mori* have included genes involved in immune responses, indicating that thermal stress can coincide with changes in defence-related molecular processes.

This association, however, does not establish that every temperature increase suppresses immunity. The direction of the response may vary with exposure duration, tissue, pathogen challenge, and developmental stage.

Future studies should distinguish basal immune competence from the response induced by infection. For example, comparing unstressed and heat-stressed larvae, with and without a defined pathogen challenge, would reveal whether thermal exposure changes infection susceptibility or the effectiveness of the induced immune response. Such experiments should include pathogen burden and survival as well as molecular expression data.

Disease-resistant silkworm strains could reduce cocoon losses and reliance on chemical treatments, but resistance must be evaluated alongside growth and silk traits. Selection for resistance that substantially reduces feeding efficiency or cocoon production may not offer an overall production advantage. Multi-trait selection is therefore central to sustainable sericulture.

3.4 Endocrine regulation, metabolism, and developmental stability

Silkworm development is regulated by endocrine signals that coordinate larval growth, moulting, metamorphosis, and tissue differentiation. Environmental stress can alter energy availability and physiological signalling, potentially disrupting the timing of these developmental processes. Changes in feeding, nutrient assimilation, and metabolic activity may consequently affect the transition between larval instars and the resources available for cocoon formation.

The relationship between stress and development is particularly important during the final larval instar, when substantial resources are allocated to silk-gland activity and cocoon construction. If thermal stress reduces feeding or increases maintenance requirements, fewer resources may remain available for silk production. Molecular studies should therefore investigate stress-responsive genes together with metabolic pathways, endocrine regulators, and developmental indicators.

Transcriptomic and metabolomic approaches can identify candidate pathways associated with developmental stability under stress. However, these candidates require functional validation and should not be treated as direct evidence of causality simply because their expression changes in stressed larvae. Controlled gene perturbation, physiological measurements, and repeatable phenotypic outcomes are needed to establish their roles.

4. Molecular Regulation of Silk-Gland Development and Silk Production

4.1 Genetic control of silk-protein biosynthesis

Silk production depends on the development and functional organization of the silkworm silk gland, a specialized organ responsible for synthesizing and secreting the proteins that form the cocoon fibre. The principal structural component is fibroin, while sericin forms a coating around the fibroin fibres. Their relative abundance, molecular composition, and organization influence important physical and processing properties of silk.

Fibroin synthesis is associated primarily with the posterior silk gland, whereas sericin production is associated predominantly with the middle silk gland. The spatial regulation of silk-protein genes reflects the differentiated functions of these glandular regions. Research on the transcriptional regulation of the silk gland has identified roles for Hox and homeodomain proteins, including *Antennapedia* and *Arrowhead*, in regulating region-specific silk-gene expression. These findings demonstrate that silk production depends not only on the presence of structural protein genes but also on the regulatory networks controlling their expression.

The major fibroin components include fibroin heavy chain, fibroin light chain, and the P25 glycoprotein, which contribute to the assembly and organization of the fibroin complex. Sericin proteins provide an adhesive coating and influence the characteristics of the resulting fibre. Changes in the expression, processing, or secretion of these proteins may affect cocoon composition and silk quality, although the relationship between transcript abundance and final fibre performance is not always direct.

This distinction is important for molecular improvement. Increased expression of a silk-protein gene does not necessarily lead to higher silk yield because production also depends on amino acid availability, cellular energy, protein folding, transport, secretion, and the functional condition of the silk gland. Selection strategies should therefore integrate gene-expression profiles with protein abundance, cocoon shell weight, shell ratio, filament length, and fibre-quality measurements.

4.2 How environmental stress affects silk biosynthesis

Silk-protein production requires substantial metabolic resources and an appropriately functioning silk gland. Heat stress can alter transcription, protein folding, cellular energy balance, and tissue development, potentially

influencing silk synthesis and secretion. Research comparing silkworms maintained at different temperatures has identified changes in genes associated with silk biosynthesis and heat-shock responses alongside changes in larval and cocoon performance.

These observations suggest that silk production should be studied as part of a broader physiological response rather than as an isolated trait. A reduction in cocoon weight following thermal stress may result from lower food intake, altered nutrient allocation, impaired silk-gland function, or a combination of these processes. Separating these effects requires simultaneous assessment of feeding behaviour, body-weight gain, silk-gland morphology, gene expression, and protein deposition.

A particularly valuable research direction is to determine whether climate-resilient strains preserve silk-gland function under repeated temperature fluctuations. Experiments should compare short heat episodes with prolonged exposure and examine whether effects differ between early and late larval instars. The inclusion of recovery periods would also reveal whether molecular changes are reversible or associated with persistent developmental impairment.

The potential role of epigenetic regulation deserves further investigation. DNA methylation, histone modifications, and non-coding RNAs could contribute to differences in stress responses, but their precise contribution to heritable climate resilience in commercial silkworm populations remains to be established. Claims that environmental exposure produces stable, beneficial epigenetic inheritance should therefore be supported by multigenerational evidence.

4.3 Molecular markers for silk quality and cocoon performance

Molecular markers can support the selection of silkworm strains with favourable combinations of stress tolerance, cocoon yield, and fibre characteristics. Candidate markers may be derived from genes involved in heat-shock responses, antioxidant defence, immune regulation, developmental control, and silk-protein synthesis. Genome-wide association studies and quantitative trait locus mapping could help identify genomic regions linked to economically important traits.

However, marker usefulness depends on repeatability across genetic backgrounds and environmental conditions. A marker associated with cocoon weight in one strain or location may not predict performance in another because genetic interactions and environmental effects can alter trait expression. Validation should therefore include multiple strains, seasons, locations, and management conditions.

Molecular-assisted selection should complement rather than replace conventional phenotyping. Candidate strains must demonstrate reliable improvements in commercial traits, acceptable disease resistance, and suitable reproductive performance. The objective is not simply to identify silkworms with strong stress-response gene expression, but to develop strains that maintain stable production under the environmental conditions encountered by farmers.

5. Sustainable Mulberry Production for Climate-Resilient Sericulture

5.1 Genetic diversity and selection of resilient mulberry cultivars

Mulberry improvement is central to climate-resilient sericulture because leaf quantity and quality influence silkworm nutrition throughout larval development. Selection of suitable cultivars should consider biomass production, leaf protein and moisture content, seasonal growth, drought response, tolerance to heat, and adaptation to local soil conditions.

Molecular tools can accelerate the identification of useful diversity within *Morus* germplasm. Genomic resources, molecular markers, transcriptomic analysis, and functional characterization can support the identification of candidate genes involved in water regulation, photosynthesis, heat response, nutrient assimilation, and plant development. The recent application of RNA sequencing to drought tolerance in *M. alba* illustrates how transcriptomic studies can help characterize stress-responsive pathways and candidate regulatory genes.

Nevertheless, molecular characterization should be linked to field performance. Greenhouse responses may not accurately predict cultivar behaviour under fluctuating temperatures, variable rainfall, different soil types, and repeated harvesting. Field trials should evaluate leaf biomass, regrowth after pruning, seasonal stability, nutrient composition, and the performance of silkworms fed with leaves from selected cultivars.

A further consideration is the distinction between plant survival and production resilience. A cultivar that survives severe drought but produces little foliage may have limited value for commercial sericulture. The most useful genotypes are those that maintain sufficient leaf yield and feeding quality while requiring fewer external inputs under locally relevant environmental constraints.

5.2 Soil health, water management, and nutrient efficiency

Sustainable mulberry production requires soil and water management practices that support stable foliage production without unnecessarily increasing environmental pressure. Organic amendments, soil-cover management, appropriate nutrient replacement, and efficient irrigation can improve resource-use efficiency when adapted to local soil and climatic conditions.

Mulberry nutrient management should be based on soil testing and crop requirements rather than the routine application of excessive fertilizer. Nutrient deficiencies may reduce leaf growth and nutritional value, whereas excessive nutrient inputs can increase production costs and contribute to environmental losses. Integrating organic amendments with appropriately balanced mineral nutrition may help maintain soil organic matter and support nutrient cycling, although outcomes depend on amendment quality, application rates, and local conditions.

Water management should similarly balance plant demand with water availability. Irrigation scheduling based on soil moisture, plant requirements, and weather conditions can reduce unnecessary water use. Mulberry cultivation systems may also benefit from mulching, improved infiltration, and measures that reduce surface evaporation, provided these practices are appropriate for the local production system.

The effects of these interventions should be assessed using both agricultural and sericultural outcomes. Measuring only mulberry biomass would not establish whether a practice improves silk productivity. Studies should also examine leaf nutrient composition, larval survival, development time, cocoon weight, and silk quality. This integrated assessment would help identify management practices that improve the performance of the entire production chain.

5.3 Biological approaches and environmentally responsible crop protection

Integrated pest and disease management can reduce unnecessary pesticide use while supporting mulberry productivity and environmental quality. Depending on the pest or pathogen, appropriate measures may include regular monitoring, sanitation, resistant cultivars, biological control, and targeted treatments when justified by local recommendations.

Crop-protection practices must also account for the sensitivity of silkworms to pesticide residues. Treatments that are acceptable for a particular mulberry pest may pose risks to larvae if contaminated foliage is subsequently used as feed. Therefore, pesticide selection, application timing, residue management, and the separation of treated foliage from silkworm feeding systems are important components of safe production.

Molecular methods may improve disease surveillance by enabling more precise identification of plant pathogens and pests. Similarly, molecular diagnostics can support the detection of certain silkworm pathogens and help distinguish infection from non-infectious causes of larval mortality. The practical value of these methods depends on diagnostic accuracy, affordability, turnaround time, and their integration into existing extension services.

Sustainable crop protection should not be defined simply as the absence of synthetic chemicals. It requires effective prevention and control of economically significant pests and diseases while minimizing risks to silkworms, beneficial organisms, farm workers, and surrounding ecosystems.

5.4 Circular bioeconomy opportunities in sericulture

Sericulture generates several potentially useful by-products, including mulberry pruning residues, silkworm litter, pupal biomass, and silk-processing residues. Appropriate recovery and reuse of these materials may contribute to circular production systems by reducing waste and returning selected nutrients to agricultural soils.

Mulberry residues may be suitable for composting or other biomass-utilization pathways, depending on their composition and contamination status. Silkworm pupae and other by-products may have value in feed, industrial, or bioprocessing applications where permitted and technically appropriate. However, such uses require assessment of hygiene, chemical residues, nutritional composition, processing requirements, and economic feasibility.

Circularity must also be evaluated in relation to the full production system. For example, converting a by-product into a useful material is not automatically environmentally beneficial if the process requires excessive energy, water, or transportation. Life-cycle assessment can help compare alternative management pathways and identify where material recovery produces meaningful environmental gains.

Integrating resource recovery with climate-resilient mulberry cultivation could help reduce input dependence and diversify farm income. Nevertheless, the benefits will vary among production regions, and the feasibility of specific interventions should be tested under local economic and environmental conditions.

6. Emerging Molecular and Omics Technologies for Sericulture Improvement

6.1 Transcriptomics and differential gene expression

Transcriptomics provides a systematic means of investigating how silkworms and mulberry plants respond to environmental stress. RNA sequencing can identify differentially expressed genes, characterize biological pathways, and reveal candidate regulators associated with stress adaptation. In *B. mori*, transcriptomic studies have already demonstrated that temperature changes affect genes associated with immunity, transcriptional regulation, silk biosynthesis, and heat-shock responses.

For mulberry, transcriptomics can help distinguish candidate pathways associated with drought tolerance, heat response, and combined stress. However, the interpretation of differentially expressed genes requires careful attention to experimental design. Tissue type, developmental stage, sampling time, stress intensity, biological replication, and sequencing depth can all influence the resulting expression profile.

A robust study should include appropriate biological replicates, clearly defined control conditions, correction for multiple statistical comparisons, and independent validation of important expression changes. Quantitative real-time PCR can be used to validate selected transcripts, although transcript validation alone does not establish the function of the encoded proteins.

An important opportunity is to compare the molecular responses of tolerant and susceptible genotypes exposed to the same environmental conditions. This comparison can help distinguish responses associated with effective tolerance from general stress responses shared by both groups. Ultimately, candidate genes must be linked to physiological performance and economically relevant traits.

6.2 Proteomics and metabolomics

Transcript abundance does not always correspond to protein abundance or activity. Translation, protein degradation, post-translational modifications, and intracellular localization can all affect the biological outcome.

Proteomics therefore provides a complementary approach for identifying proteins involved in stress tolerance, silk biosynthesis, and plant adaptation.

Combined transcriptomic and proteomic analysis has been applied to *M. alba* under simultaneous drought and heat stress, demonstrating the value of investigating gene expression and protein-level responses together. Such approaches can help identify regulatory networks that are not readily understood from transcript data alone.

Metabolomics can further characterize changes in sugars, amino acids, organic acids, antioxidants, and other metabolites associated with stress adaptation. In mulberry, these measurements may clarify whether a stress-tolerant cultivar maintains nutrient availability for feeding larvae. In silkworms, metabolite profiles can reveal shifts in energy use, amino acid metabolism, and resource allocation during development.

The strongest approach combines these datasets with phenotypic measurements. For example, a candidate molecular signature associated with drought tolerance should be examined alongside leaf biomass, water-use efficiency, nutrient composition, and subsequent silkworm performance. Without this connection, multi-omics studies may generate extensive lists of molecular changes without establishing their practical value.

6.3 Genome-wide association studies and molecular-assisted breeding

Genomic approaches can identify variation associated with economically important traits and help breeders select promising parental lines. Genome-wide association studies can examine relationships between genetic variants and traits such as thermal tolerance, larval survival, cocoon weight, silk content, and developmental duration. Quantitative trait locus mapping can similarly identify genomic regions associated with measurable differences among segregating populations.

The usefulness of these approaches depends on adequate population size, genetic diversity, reliable phenotyping, and appropriate statistical analysis. Population structure and relatedness must be accounted for to reduce false associations. Candidate markers should subsequently be validated in independent populations and under different environmental conditions.

For mulberry, molecular-assisted breeding could support the selection of cultivars combining drought tolerance, heat resilience, productive regrowth, and suitable leaf quality. For silkworms, marker-assisted selection may help combine stress tolerance with disease resistance and favourable cocoon characteristics.

Genomic selection is another potential strategy, particularly where important traits are controlled by many genes with relatively small individual effects. By estimating breeding values from genome-wide marker information, this approach may accelerate selection when supported by sufficiently large and representative training populations. Its practical effectiveness in sericulture will depend on the availability of standardized phenotypic data, genotyped breeding material, and validation across production environments.

6.4 Functional genomics and gene-editing approaches

Functional genomics helps determine whether candidate genes contribute directly to stress tolerance or silk production. RNA interference can reduce the expression of selected genes, while transgenic approaches and genome-editing technologies can be used to investigate gene function under controlled experimental conditions. Functional evidence is especially valuable when transcriptomic studies identify several correlated candidate genes but cannot establish which are necessary for the observed phenotype. For example, targeted investigation of heat-shock proteins has shown that different members of the HSP family can make unequal contributions to silkworm survival during thermal stress.

Gene editing may eventually support the development of silkworm strains with improved stress resilience or altered silk properties. However, the translation of experimental modifications into commercial sericulture requires assessment of developmental stability, reproductive performance, genetic background, unintended effects, biosafety, regulatory requirements, and public acceptance.

For this reason, gene editing should be considered one component of a broader breeding strategy rather than a replacement for conventional selection. Where suitable natural variation already exists, conventional or marker-assisted breeding may offer a more accessible route to improvement. Functional genomics can strengthen both approaches by clarifying the mechanisms underlying desirable traits.

6.5 Epigenomics, microbiome analysis, and systems biology

Epigenomics investigates molecular processes that influence gene activity without necessarily changing the underlying DNA sequence. DNA methylation, histone modifications, chromatin accessibility, and non-coding RNAs may contribute to variation in stress responses. These mechanisms are promising areas for research into how silkworms and mulberry plants respond to fluctuating environmental conditions.

However, evidence of an epigenetic response during stress should not automatically be interpreted as evidence of a stable, inherited adaptation. Longitudinal studies and multigenerational experiments are needed to distinguish transient responses from persistent changes that influence subsequent generations.

The microbiome represents another potentially important component of the sericulture system. Microorganisms associated with the silkworm gut, mulberry phyllosphere, and soil may influence nutrient availability, digestion, immunity, and plant health. Environmental stress could alter these microbial communities, potentially modifying interactions between host plants and silkworms.

Nevertheless, associations between microbial composition and productivity do not establish causation. Microbiome research should combine community profiling with functional measurements, controlled inoculation experiments where appropriate, and validation under realistic rearing conditions.

Systems biology provides a framework for integrating genomic, transcriptomic, proteomic, metabolomic, microbiome, and environmental data. Rather than examining each dataset independently, integrated analysis can identify interactions among plant nutrition, insect stress responses, immune regulation, and silk production. The ultimate objective is to determine which molecular and ecological relationships consistently explain variation in sericultural performance.

7. Integrating Molecular Findings with Practical Sericulture Management

Molecular technologies are most useful when their results can be translated into decisions that improve productivity, reduce losses, or strengthen environmental sustainability. The translation process should connect molecular discovery with genotype selection, cultivation practices, rearing management, and measurable production outcomes.

At the mulberry level, molecular screening can identify promising candidate cultivars, but these cultivars must be evaluated in field trials for leaf yield, nutritional quality, seasonal stability, and water requirements. At the silkworm level, molecular markers and gene-expression profiles can help prioritize strains for testing, but final selection should depend on larval performance, disease resistance, cocoon traits, and silk quality.

Environmental monitoring can strengthen this process by recording temperature, relative humidity, soil moisture, and other relevant conditions during cultivation and rearing. Linking these measurements with molecular and phenotypic data may reveal the environmental conditions under which particular genotypes perform most consistently.

A practical framework should therefore proceed through four connected stages: molecular discovery, controlled validation, field or rearing-house evaluation, and farmer-level assessment. Each stage should have clearly defined performance criteria. Candidate genes should be validated experimentally, selected genotypes should be assessed across relevant environments, and recommended management practices should be tested for affordability and operational feasibility.

Extension services and farmer participation are essential to this translation. Farmers can identify recurring production constraints, seasonal differences, and practical barriers that may not be evident in controlled experiments. Participatory trials can help determine whether a promising intervention remains effective under routine farm conditions.

The success of integrated sericulture should ultimately be assessed through a balanced set of indicators, including cocoon yield, silk quality, larval survival, leaf productivity, water and nutrient efficiency, disease incidence, production costs, and environmental impacts. A strategy that increases yield but substantially raises resource consumption or production costs may not provide a durable advantage. Conversely, a modest improvement in yield stability combined with reduced losses and lower input requirements may offer meaningful long-term benefits.

8. Research Gaps and Future Perspectives

Despite advances in silkworm molecular biology and mulberry stress physiology, several important gaps limit the translation of molecular discoveries into climate-resilient sericulture.

First, many molecular investigations focus on a single stressor, whereas commercial production frequently involves interacting environmental pressures. Experiments that examine heat, drought, humidity, and nutritional limitation separately may not adequately predict performance under combined stress. Factorial experiments should therefore investigate single and combined stress treatments, including recovery periods and repeated exposure, to determine how stress interactions influence development and productivity.

Second, molecular responses are often examined without sufficiently detailed measurements of economic traits. Differential gene expression, protein abundance, and metabolite changes can identify potential mechanisms, but their practical value depends on whether they predict improvements in survival, cocoon yield, shell ratio, filament characteristics, or production stability. Future studies should prioritize molecular indicators that demonstrate reproducible relationships with measurable performance.

Third, the interaction between mulberry nutritional quality and silkworm molecular responses remains insufficiently integrated into a single research framework. Drought may alter leaf chemistry, which can subsequently affect feeding, digestion, metabolism, immune function, and silk-gland activity. Studies that combine leaf nutrient profiling with silkworm transcriptomics, metabolomics, and cocoon measurements could clarify these relationships.

Fourth, molecular findings require stronger validation across genetic backgrounds and production environments. Candidate markers identified in one cultivar or silkworm strain may not remain predictive under different environmental conditions. Multi-location trials, independent validation populations, and standardized stress protocols are needed to establish the reliability of molecular-assisted selection.

Fifth, the economic and environmental implications of advanced molecular technologies require further assessment. High-throughput sequencing, genome editing, and integrated multi-omics may offer substantial scientific value, but their implementation may be constrained by cost, technical expertise, laboratory infrastructure, and access to breeding material. Research should therefore compare advanced methods with more accessible alternatives, including conventional selection, marker-assisted breeding, improved irrigation, and better rearing-house management.

Sixth, the potential contribution of epigenetic regulation and microbial communities requires carefully designed functional studies. These fields may reveal mechanisms that complement genetic selection, but correlations should

not be treated as proof of causal effects. Longitudinal, multigenerational, and experimentally validated investigations are needed before such findings can be incorporated into practical recommendations. Finally, sustainability assessments should extend beyond biological performance. Life-cycle assessment, resource-use accounting, and economic analysis can help determine whether new cultivars, molecular tools, and management practices provide net benefits under different production systems. Greater collaboration among molecular biologists, entomologists, plant breeders, climate scientists, sericulture extension specialists, and farmers will be necessary to achieve this objective.

Table 1. Major Molecular Targets and Their Potential Applications in Climate-Resilient Sericulture

Molecular or biological target	Research focus	Potential application	Key validation requirement
Heat-shock proteins, including HSP70 and HSP90	Protein protection and thermal adaptation	Selection of thermotolerant silkworm strains	Survival, growth, and cocoon performance under heat stress
Antioxidant defence pathways	Redox regulation and oxidative damage	Identification of stress-resilient plant and insect genotypes	Enzyme activity, oxidative damage, and physiological performance
Immune-related genes	Host defence and pathogen response	Improved disease-resistance screening	Pathogen burden, survival, and cocoon yield
Silk-protein genes and regulatory factors	Fibroin and sericin synthesis	Improvement of silk yield and fibre properties	Protein abundance, shell ratio, and filament quality
Drought-response genes in mulberry	Water regulation and stress adaptation	Selection of drought-resilient mulberry cultivars	Leaf yield, water-use efficiency, and nutritional quality
Genomic markers and quantitative trait loci	Genetic variation associated with production traits	Marker-assisted selection and breeding	Independent population and multi-environment validation
Epigenetic regulators	Regulation of stress-responsive gene activity	Investigation of persistent stress-response variation	Longitudinal and multigenerational validation
Host-associated microbial communities	Nutrition, immunity, and plant–insect interactions	Development of biologically informed management strategies	Functional experiments and field validation

Note: These are research priorities and potential applications, not a claim that every target is already validated as a reliable predictor of commercial performance.

Table 2. Proposed Research Priorities for Translating Molecular Discoveries into Sustainable Sericulture

Research priority	Recommended approach	Expected outcome
Combined climate-stress assessment	Factorial experiments involving heat, drought, and relevant humidity conditions	Identification of stress interactions and resilient genotypes
Integrated plant–insect analysis	Combine mulberry leaf chemistry with silkworm molecular and physiological measurements	Improved understanding of host-plant effects on silk productivity
Multi-omics integration	Link transcriptomics, proteomics, metabolomics, and phenotypic data	Identification of validated candidate pathways and biomarkers
Molecular-assisted breeding	Combine genomic markers with conventional selection	More efficient identification of productive, resilient cultivars and strains
Field validation	Conduct replicated, multi-location trials across seasons	Evidence of stability under realistic production conditions
Sustainable resource management	Evaluate irrigation, nutrient management, and biological crop protection	Reduced resource losses and improved production efficiency
Microbiome and epigenetic research	Conduct functional and longitudinal experiments	Better understanding of non-genetic and microbial contributions to resilience
Farmer-level implementation	Test selected interventions with producers and extension services	Assessment of affordability, usability, and adoption potential

These priorities provide a pathway for moving from molecular discovery to practical implementation. The most immediate opportunities lie in integrating existing stress-response knowledge with systematic phenotyping, improved mulberry cultivation, and better control of the silkworm rearing environment. More advanced technologies can then be directed toward the biological questions that remain unresolved.

9. CONCLUSION

The future of sericulture depends on the ability to maintain productive mulberry cultivation and silkworm development under increasingly variable environmental conditions. Molecular biology provides valuable tools for understanding stress responses, identifying candidate genes, and investigating the regulation of immunity, metabolism, silk-gland development, and silk-protein biosynthesis. At the same time, climate-resilient mulberry cultivars, efficient water and nutrient management, integrated disease control, and responsible resource utilization remain essential components of a sustainable production system.

Current evidence demonstrates that thermal stress can alter silkworm gene expression and cocoon performance, while combined drought and heat can trigger complex molecular responses in mulberry. These findings establish a foundation for integrating molecular research with agricultural and sericultural improvement. However, the identification of stress-responsive genes should be regarded as an initial step rather than a direct guarantee of improved productivity. Candidate genes, molecular markers, and breeding materials must be validated against economically important traits and evaluated across relevant environmental conditions. An integrated approach combining genomics, transcriptomics, proteomics, metabolomics, functional validation, and multi-environment phenotyping offers the strongest pathway toward identifying resilient plant and insect genotypes. Its effectiveness will depend on the quality of experimental design, the reproducibility of molecular associations, and the successful translation of research findings into affordable farm-level interventions. Future research should place particular emphasis on combined environmental stresses, plant–silkworm nutritional interactions, long-term performance stability, and the environmental and economic consequences of new technologies. Ultimately, climate-resilient sericulture should be understood as a coordinated biological and agricultural system rather than an isolated breeding or molecular research problem. Connecting molecular discoveries with sustainable mulberry production, improved silkworm management, and farmer-oriented innovation can contribute to more reliable silk production, reduced resource dependence, and stronger livelihood resilience under changing environmental conditions.

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