

ADVANCES IN MOLECULAR BIOTECHNOLOGY, GENOMICS AND GENE EXPRESSION PROFILING FOR SILKWORM DISEASE RESISTANCE, SILK QUALITY IMPROVEMENT AND SUSTAINABLE SERICULTURE DEVELOPMENT

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ABSTRACT

Sericulture is an economically important agro-based industry whose productivity depends on silkworm health, genetic potential, host-plant quality, disease management, and the efficiency of silk biosynthesis. The domesticated silkworm, *Bombyx mori*, has become a valuable model for molecular genetics and biotechnology because of its established genomic resources, experimentally tractable biology, and specialized silk-producing glands. Nevertheless, viral, bacterial, fungal, and microsporidian diseases continue to cause substantial production losses, while the demand for improved silk quality and environmentally sustainable production creates additional breeding and management challenges. This review critically examines advances in molecular biotechnology, genomics, gene expression profiling, molecular diagnostics, and functional genetics for improving disease resistance and silk quality. Particular attention is given to host–pathogen interactions, antiviral RNA interference, immune signalling, candidate resistance genes, transcriptomic analysis, molecular marker-assisted selection, genome editing, and the genetic regulation of fibroin and sericin biosynthesis. The potential of proteomics, metabolomics, epigenomics, and integrated multi-omics to identify biomarkers and explain complex production traits is also discussed. Furthermore, the review considers how molecular diagnostics can support early pathogen detection and how conventional breeding can be integrated with modern biotechnological approaches to improve resistance without compromising larval development, cocoon yield, or fibre characteristics. Major challenges include the limited translation of laboratory findings into commercial strains, the complexity of quantitative resistance, possible fitness trade-offs, and the need for reliable multi-environment validation. A framework is proposed for connecting molecular discovery, functional validation, breeding, disease surveillance, and sustainable farm-level implementation. Integrating these approaches could strengthen silkworm health, improve silk quality, reduce avoidable production losses, and support the long-term sustainability of sericulture.

KEYWORDS: *Bombyx mori*; molecular biotechnology; genomics; gene expression profiling; disease resistance; silk fibroin; sericin; molecular diagnostics; sustainable sericulture.

1. INTRODUCTION

Sericulture occupies a distinctive position at the intersection of agriculture, insect biology, biotechnology, and rural enterprise. The production of natural silk depends on the successful interaction of silkworm genetics, host-plant nutrition, environmental conditions, disease prevention, and rearing management. Among commercially cultivated silk-producing insects, the domesticated silkworm, *Bombyx mori*, is particularly important because of its long history of domestication, extensive genetic resources, and capacity to produce silk fibres with valuable mechanical and processing properties. Its well-characterized genome and established experimental systems have also made it a major model for investigating insect development, immunity, gene regulation, and biological protein production [1-2]. Despite substantial improvements in rearing practices and silkworm breeding, disease remains a major constraint on production. Viral infections, bacterial diseases, fungal pathogens, and microsporidian parasites can impair larval growth, increase mortality, reduce cocoon formation, and compromise the quality of harvested cocoons. Among the important pathogens are *Bombyx mori* nucleopolyhedrovirus (BmNPV), *Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV), and *Nosema bombycis*, the microsporidian associated with pébrine disease. Their biological differences create distinct challenges for diagnosis and control, making it necessary to develop pathogen-specific approaches rather than relying on a single universal disease-management strategy. Recent research has increasingly investigated these diseases through molecular pathology, genomics, and host–pathogen interaction studies. Traditional disease surveillance has relied heavily on visual symptoms, microscopic examination, sanitation, and established rearing protocols [3-5]. These approaches remain essential, but they may not always identify infections at an early stage or distinguish closely related pathogens. Molecular diagnostics based on pathogen-specific nucleic acid sequences can improve the sensitivity and specificity of detection, particularly where pathogens are difficult to identify through conventional methods alone. Polymerase

chain reaction (PCR), reverse-transcription PCR (RT-PCR), quantitative PCR (qPCR), and emerging isothermal amplification approaches offer opportunities for more rapid surveillance and improved disease management. Their practical effectiveness, however, depends on validated assays, suitable sampling, contamination control, and access to appropriate laboratory or field-testing infrastructure. Advances in molecular biotechnology have also changed the prospects for improving silkworm disease resistance. Genomic resources, transcriptome sequencing, gene-expression analysis, molecular markers, RNA interference, transgenic technologies, and CRISPR/Cas-based genome editing provide complementary methods for identifying and investigating genes involved in immunity and disease susceptibility. These technologies can help distinguish candidate resistance mechanisms, characterize genetic variation among silkworm strains, and support the development of improved breeding populations. Nevertheless, a gene that responds to pathogen infection is not necessarily a gene that confers effective resistance [6-7]. Molecular associations require functional validation and evaluation against measurable outcomes, including pathogen burden, larval survival, developmental stability, cocoon yield, and silk quality.

The improvement of silk quality presents a related but distinct challenge. Silk fibroin forms the principal structural component of the fibre, while sericin contributes to the coating that binds fibroin filaments. Their synthesis depends on specialized silk-gland tissues, coordinated transcriptional regulation, amino acid availability, protein processing, and secretion. Molecular studies of silk-gland development have revealed regulatory mechanisms that control the spatial expression of silk-related genes, creating opportunities to investigate variation in cocoon composition and fibre characteristics. A central objective of modern sericulture research is therefore to integrate disease resistance and silk quality within a unified improvement strategy [8]. Selecting a strain solely for resistance may be counterproductive if the trait is accompanied by slower development, reduced fecundity, lower cocoon weight, or undesirable fibre properties. Similarly, improving silk characteristics without accounting for disease susceptibility could leave production vulnerable to substantial losses. Molecular biotechnology should consequently support multi-trait improvement, combining resistance, productivity, fibre quality, and reproductive fitness.

This review examines the principal molecular and biotechnological approaches available for achieving these goals. It discusses the genetic and molecular foundations of silkworm disease resistance, advances in pathogen detection, the regulation of silk-protein biosynthesis, and the applications of genomics and functional genetics in breeding [9]. It also evaluates the limitations of current evidence and proposes an integrated research framework for translating molecular discoveries into reliable and sustainable sericulture practices.

2. Major Silkworm Diseases and Their Molecular Basis

2.1 Viral diseases and host–virus interactions

Viral diseases are among the most serious biological constraints in commercial silkworm rearing because infection can interfere with cellular function, larval development, and cocoon formation. BmNPV is a major pathogen associated with nuclear polyhedrosis, whereas BmCPV causes cytoplasmic polyhedrosis. These viruses differ in their genome organization, replication strategies, cellular interactions, and disease manifestations. Consequently, understanding their molecular biology is essential for developing effective diagnostic tools and resistance-improvement strategies.

BmNPV infection involves interactions between viral entry, replication, host cellular machinery, and antiviral defence. Viral replication can alter host transcription, metabolism, protein synthesis, and cellular survival pathways. The severity of infection depends on several factors, including viral strain, infectious dose, host genotype, developmental stage, and environmental conditions. Molecular studies have therefore focused on identifying host factors that facilitate viral replication and defence mechanisms that restrict infection [10-12]. One important component of insect antiviral defence is RNA interference (RNAi). This mechanism can recognize double-stranded RNA and process it into small interfering RNAs that guide sequence-specific degradation of complementary RNA molecules. In silkworms, RNAi-related processes have been investigated as part of the response to viral infection and as experimental tools for examining gene function. However, the effectiveness of antiviral RNAi depends on the virus, the target sequence, the delivery method, and the biological context. Its practical use in commercial sericulture requires further validation regarding delivery efficiency, durability, safety, and cost.

Other host pathways, including apoptosis regulation, cellular stress responses, and innate immune signalling, may also influence viral infection outcomes. These mechanisms can interact in complex ways, and viral proteins may interfere with several host defence processes simultaneously. As a result, resistance may depend on the coordinated activity of multiple genes rather than a single molecular determinant. Recent reviews of silkworm disease research highlight the importance of integrating mechanistic studies of host immunity with pathogen biology and functional genetic validation [13]. For breeding purposes, the most valuable traits are those that reduce disease-associated losses without compromising normal development. Research should therefore distinguish between resistance, in which the host restricts pathogen infection or replication, and tolerance, in which the host maintains performance despite infection. This distinction is particularly important when evaluating candidate genotypes under controlled pathogen challenge and subsequent field or rearing-house conditions.

2.2 Bacterial diseases and molecular defence mechanisms

Bacterial infections can affect silkworm health through tissue damage, toxin production, systemic infection, or disruption of normal physiological functions. The outcome depends on the bacterial species, strain, route of exposure, larval condition, and environmental circumstances. Poor sanitation, contaminated rearing equipment,

and unsuitable environmental conditions can increase disease risks, although the contribution of each factor differs among pathogens and production systems.

Molecular investigation of bacterial disease involves identifying the pathogen and examining the host responses triggered by infection. Candidate processes include antimicrobial peptide production, recognition of microbial components, activation of cellular defence mechanisms, and regulation of immune signalling. Insect antimicrobial peptides can contribute to the control of particular microorganisms, but their activity varies according to peptide structure, pathogen susceptibility, and the physiological environment [14]. Gene-expression profiling can reveal changes in immune-related transcripts following bacterial challenge. Nevertheless, differential expression alone does not prove that a particular gene protects the host. Functional studies using gene silencing, overexpression, or other appropriate perturbation approaches are needed to determine whether a candidate factor contributes directly to resistance. Researchers should also evaluate whether immune activation is accompanied by physiological costs that influence growth or silk production. Integrated disease management remains essential because molecular resistance is unlikely to eliminate all risks associated with bacterial infection. Clean rearing facilities, appropriate handling of larvae, effective waste management, and rapid identification of disease outbreaks should continue alongside breeding and molecular research. Where treatments are considered, their suitability for silkworms and their potential effects on residues, beneficial organisms, and the surrounding environment should be assessed carefully.

2.3 Fungal infections and host defence

Fungal pathogens can cause substantial losses in silkworm production, particularly when environmental and management conditions favour infection. Muscardine disease, commonly associated with entomopathogenic fungi such as *Beauveria bassiana*, is an important example. Infection may involve the attachment of fungal propagules to the insect cuticle, penetration of the external barrier, and subsequent colonization of host tissues.

The insect cuticle provides an initial physical and biochemical barrier against fungal invasion. Once this barrier is breached, immune recognition, cellular defence, melanization, and other physiological processes can influence the progression of infection. Molecular studies can help characterize these responses and identify differences among silkworm genotypes in their ability to limit fungal growth. The relationship between humidity and fungal disease is particularly relevant to rearing management. Moisture conditions can influence fungal survival and transmission, but the effect varies with the pathogen and the rearing environment. Molecular resistance studies should therefore be designed alongside controlled environmental measurements to avoid attributing differences in infection solely to genetic variation when exposure conditions may also differ [15]. Fungal resistance should be evaluated using a combination of survival, pathogen load, developmental performance, and cocoon traits. The objective is to identify genotypes that maintain productive performance while limiting disease progression, rather than those that show a molecular response without a meaningful reduction in disease-associated losses.

2.4 Microsporidian infection and pébrine disease

Pébrine disease, caused by *Nosema bombycis*, is particularly important because the pathogen can be transmitted through infected silkworm eggs and can persist across generations. Infection may interfere with larval development, reduce survival, and compromise cocoon production. Its transmission biology makes disease prevention and the screening of breeding material especially important.

Molecular diagnostics provide opportunities to identify *N. bombycis* more reliably than symptom-based inspection alone. PCR-based assays and related nucleic acid detection methods can support screening of silkworm material and help identify infections that might otherwise remain undetected. Their effectiveness depends on appropriate sampling, assay sensitivity, and validated laboratory procedures [16]. Molecular studies of pébrine also offer opportunities to examine host–parasite interactions, pathogen variation, and potential resistance-associated genes. However, reducing pathogen burden in an experimental setting does not automatically demonstrate complete resistance or prevent vertical transmission. Long-term evaluation should consider reproductive performance, infection status across generations, and the reliability of disease screening under practical production conditions. Given the potential for transgenerational spread, molecular approaches should complement established preventive practices, including egg screening, hygiene, isolation of affected material, and appropriate rearing management. Recent research emphasizes that improved understanding of parasite biology and host responses must be translated into dependable prevention and detection strategies.

Table 1. Major Silkworm Diseases and Opportunities for Molecular Intervention

Disease or pathogen	Major research focus	Relevant molecular approaches	Potential application
BmNPV infection	Viral replication and host antiviral responses	RNAi analysis, transcriptomics, functional genetics	Identification of resistance-associated pathways
BmCPV infection	Virus–host interactions and infection mechanisms	Molecular detection, gene-expression analysis	Improved diagnosis and host-response characterization
Bacterial diseases	Pathogen identification and innate immunity	PCR, antimicrobial peptide profiling, transcriptomics	Earlier diagnosis and resistance screening

Muscardine disease	Fungal invasion and host defence	Pathogen-specific assays, immune-gene profiling	Improved surveillance and resistance evaluation
Pébrine disease	Microsporidian infection and transmission	PCR-based detection, pathogen genotyping, host genetics	Breeding-stock screening and investigation of resistance
Mixed or uncertain infections	Identification of multiple pathogens	Validated multiplex assays and sequencing-based methods	More accurate disease diagnosis

Note: The molecular approaches listed are potential research or diagnostic tools. Their suitability depends on pathogen-specific validation and the intended application.

3. Genomics and Genetic Resources for Silkworm Improvement

3.1 From genome sequencing to functional genomics

The development of genomic resources for *B. mori* has transformed the investigation of silkworm biology. Genome sequencing, molecular linkage maps, gene annotation, and comparative genomic analysis have provided a foundation for studying development, immunity, silk biosynthesis, and domestication. These resources allow researchers to investigate biological traits at a scale that was previously difficult to achieve using conventional genetic methods alone. Structural genomics characterizes the organization and variation of the genome, while functional genomics examines how genes and regulatory elements contribute to biological processes. In sericulture, these complementary approaches can identify candidate genes associated with disease resistance, larval growth, reproductive performance, and silk production [17].

Genomic comparisons among silkworm strains can reveal sequence variation that may be associated with differences in resistance or productivity. However, the interpretation of such variation requires care. A genetic variant may be correlated with a trait because it is linked to another functional variant, or because the population contains underlying genetic structure. Genome-wide association studies should therefore account for population structure and relatedness, and promising variants should be tested in independent populations. The application of genomics to breeding is strongest when genetic data are integrated with standardized phenotypic information. For example, resistance-associated genomic regions should be evaluated alongside pathogen burden, larval survival, cocoon shell ratio, and developmental stability. This integration can distinguish useful genetic markers from associations that have limited predictive value in commercial populations.

3.2 Comparative genomics and silkworm diversity

Silkworm breeding depends on maintaining and utilizing genetic diversity. Domesticated strains differ in their developmental characteristics, cocoon traits, environmental responses, and susceptibility to disease. Wild relatives and other genetic resources may provide additional variation, although their incorporation into breeding programmes requires careful assessment of compatibility and desirable traits [18]. Comparative genomics can help identify conserved and divergent genes among silkworm populations. Such comparisons may reveal candidate regions associated with domestication, silk production, immunity, or adaptation to particular rearing conditions. Population genomics can further clarify genetic relationships and help breeders manage diversity within breeding collections. However, the identification of a gene that differs between resistant and susceptible strains is only the beginning of the investigation. Functional validation is needed to establish its biological role, and breeding studies must determine whether the favourable trait can be transferred without introducing undesirable characteristics. Conservation of genetic resources is therefore an important part of molecular sericulture. Maintaining diverse silkworm lines provides material for future breeding, allows researchers to investigate alternative resistance mechanisms, and reduces the risks associated with excessive reliance on a narrow genetic base.

3.3 Molecular markers and marker-assisted selection

Molecular markers can support selection by identifying genetic regions associated with desirable traits. In silkworm breeding, potential targets include disease resistance, developmental duration, cocoon weight, shell ratio, filament length, and silk quality. Marker-assisted selection can be particularly useful when the phenotype is difficult to measure reliably, when evaluation requires pathogen exposure, or when selection decisions need to be made early in development [19]. The effectiveness of marker-assisted selection depends on the strength and stability of the marker–trait association. Traits controlled by a single major-effect gene may be more straightforward to select than complex traits influenced by numerous genes and environmental conditions. Disease resistance and silk quality can involve multiple interacting mechanisms, making validation across genetic backgrounds especially important.

Marker-assisted breeding should not be viewed as a substitute for conventional selection. Instead, molecular markers can improve the efficiency of identifying promising individuals, while phenotypic evaluation confirms that the selected material performs as expected. Breeding programmes should also monitor fertility, growth, cocoon characteristics, and other traits that may be affected by selection.

3.4 Genomic selection for complex production traits

Genomic selection uses genome-wide marker information to estimate the genetic merit of individuals for traits that may be influenced by many genes. It may be useful for complex outcomes such as disease resistance, silk yield, and environmental stability, where individual markers may explain only a small proportion of the observed

variation. Successful implementation requires sufficiently large training populations with reliable genotypes and phenotypes. Prediction accuracy must be evaluated in populations that are independent of the training data and, ideally, under different environmental conditions. If a prediction model is trained using a narrow genetic background, its performance may decline when applied to unrelated strains [20]. In sericulture, genomic selection could potentially accelerate the identification of breeding lines that combine resistance with favourable cocoon and silk traits. Nevertheless, its practical value depends on the cost of genotyping, the availability of well-characterized breeding populations, and the capacity to maintain consistent phenotypic records. These considerations are particularly important for small and medium-sized breeding programmes.

4. Gene Expression Profiling and Molecular Regulation of Disease Resistance

4.1 Transcriptomics as a tool for identifying resistance mechanisms

Gene expression profiling provides a means of examining how silkworm genes respond to infection, environmental stress, and changes in physiological condition. RNA sequencing enables the simultaneous measurement of thousands of transcripts, allowing researchers to identify differentially expressed genes, characterize enriched biological pathways, and investigate interactions among regulatory networks [21]. In disease research, transcriptomic comparisons can be performed between infected and uninfected larvae, resistant and susceptible strains, or individuals exposed to different pathogen loads. These comparisons can identify candidate genes associated with immune recognition, antiviral defence, apoptosis, metabolism, cellular stress, and tissue repair. However, the resulting gene lists must be interpreted in relation to the experimental conditions and the biological stage at which samples were collected.

A gene may show increased expression because it contributes to resistance, because the host is responding to tissue damage, or because the infection has progressed to a more severe stage. Similarly, reduced expression may indicate suppression of a defence pathway, a change in tissue composition, or a difference in developmental timing. Consequently, transcriptomic studies should include biological replication, appropriate controls, clearly defined infection conditions, and suitable statistical correction for multiple testing. The interpretation of transcriptomic data is strengthened by independent validation. Quantitative real-time PCR can confirm selected expression patterns, while functional experiments can determine whether a candidate gene contributes to resistance. Where feasible, transcriptomic findings should also be integrated with pathogen quantification, protein measurements, and phenotypic outcomes.

4.2 Innate immune signalling pathways

Silkworm immunity involves molecular systems that recognize pathogens and coordinate cellular and biochemical defence responses. Important pathways investigated in insect immunity include Toll, immune deficiency (IMD), Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase (MAPK), and melanization-related mechanisms. Their specific contributions vary according to the pathogen and the biological context [22]. Toll-associated signalling is linked to the regulation of immune responses against particular microbial challenges, while IMD-related processes contribute to the regulation of antimicrobial defence. JAK/STAT and MAPK signalling participate in multiple physiological functions, including immune responses, cellular regulation, and stress signalling. These pathways should not be treated as independent switches because cross-talk and shared regulatory components can influence the overall response. Melanization provides another component of insect defence, involving enzymatic processes that generate melanin around pathogens or damaged tissues. Although melanization can contribute to pathogen restriction, excessive or poorly regulated activation may also impose physiological costs. Understanding how these responses are controlled could help identify genetic variation that supports effective defence without disrupting normal development.

The application of pathway analysis to silkworm disease research can help prioritize candidate genes and regulatory networks for functional investigation. However, pathway enrichment indicates statistical overrepresentation of annotated genes, not direct proof that the pathway is activated or that it determines resistance. Experimental validation remains necessary to establish the direction and functional significance of the response.

4.3 Antimicrobial peptides and immune effectors

Antimicrobial peptides are small molecules that contribute to the defence of insects against particular microorganisms. In silkworms, their production and regulation can provide useful indicators of immune activity. Their effectiveness depends on peptide sequence, concentration, microbial susceptibility, and the conditions under which host–pathogen interactions occur [24]. Gene-expression studies can identify antimicrobial peptide genes that respond to infection, but expression alone does not establish their protective value. Some peptides may be induced too late to prevent substantial infection, while others may have activity against certain microbial groups but limited effectiveness against others. Functional assays should therefore combine expression analysis with appropriate antimicrobial tests and *in vivo* validation where feasible. Breeding programmes could investigate genetic variation in immune effectors and their regulatory elements. However, selecting for elevated baseline immune activity without considering its metabolic costs may not improve overall productivity. A desirable strain should respond effectively to infection while maintaining feeding, growth, reproduction, and cocoon formation.

4.4 RNA interference and functional gene validation

RNA interference is widely used as a functional genomics approach to investigate gene function in silkworms. By reducing the expression of a selected transcript, researchers can examine whether the corresponding gene

contributes to immunity, development, metabolism, or silk production. This approach can help distinguish candidate genes that merely correlate with a trait from those that have a functional influence. In antiviral research, RNAi also provides a potential route for sequence-specific suppression of viral transcripts. Nevertheless, its effectiveness depends on the target, delivery system, stability of the RNA molecules, and biological accessibility of the infected tissues. The results of laboratory experiments should not be assumed to translate directly into commercial disease control. Gene-silencing studies require suitable negative controls, confirmation of target knockdown, assessment of unintended effects, and replication across independent experiments. Where the target gene has an important developmental role, reduced survival or altered cocoon production may result from the disruption of normal physiology rather than from the intended disease-resistance mechanism. For breeding applications, RNAi findings should be combined with genomic analysis and other functional approaches to determine whether naturally occurring variation in the target gene could be used for selection. This would help connect mechanistic discovery with practical genetic improvement.

5. Molecular Diagnostics and Precision Disease Surveillance

5.1 PCR and quantitative PCR

PCR-based diagnostic methods have become important tools for identifying silkworm pathogens. Conventional PCR amplifies selected DNA sequences, whereas RT-PCR is used when RNA must first be converted into complementary DNA. Quantitative PCR enables monitoring of amplification in real time and can provide estimates of target nucleic acid abundance when appropriately validated standards and controls are used.

For RNA viruses, the reverse-transcription step is essential because the viral genome is RNA rather than DNA. For DNA viruses and other DNA-containing pathogens, suitable DNA-based assays can be employed. Assay design must account for pathogen diversity, sequence conservation, sample quality, and the possibility of contamination. Quantitative results require particular care in interpretation. A lower pathogen-associated signal may indicate reduced pathogen burden, but the result depends on sample preparation, assay efficiency, the target sequence, and the normalization method. Diagnostic thresholds should be established through validation rather than selected arbitrarily [25]. Molecular diagnostics are especially valuable when combined with routine surveillance and established preventive practices. Early identification of infected material can help reduce the spread of disease, improve decisions about isolation, and strengthen the screening of breeding stocks.

5.2 Isothermal amplification and field-oriented diagnostics

Although PCR is sensitive and versatile, it requires temperature-control equipment and reliable laboratory procedures. Isothermal amplification methods, including loop-mediated isothermal amplification (LAMP), operate at a relatively constant temperature and may be useful in settings where conventional thermocyclers are not readily available.

LAMP and related technologies can support more accessible pathogen detection, but their suitability depends on assay specificity, sensitivity, sample preparation, reagent stability, and the interpretation of results. Poorly designed assays may produce nonspecific amplification, while inadequate contamination control can generate false-positive results. The development of field-oriented diagnostic systems should therefore include validation using known positive and negative samples, assessment of performance across pathogen variants, and comparison with established reference methods. Where diagnostic decisions have substantial economic consequences, confirmatory testing may be appropriate.

Portable molecular testing could improve disease surveillance in sericulture regions where access to centralized laboratories is limited. However, deployment should consider the total cost of equipment, reagents, training, sample transport, and quality assurance rather than focusing only on the nominal cost of an individual test.

5.3 Sequencing-based pathogen identification

Sequencing approaches can be useful when conventional diagnostic assays fail to identify an organism or when mixed infections are suspected. Targeted sequencing can characterize selected genomic regions, while broader sequencing approaches may reveal unexpected pathogens or provide information about genetic variation among isolates.

Metagenomic analysis offers the possibility of examining multiple microbial agents within a sample without requiring a separate pathogen-specific assay for each organism. Nevertheless, the detection of a sequence does not automatically establish that the organism is causing the observed disease. Interpretation must consider background contamination, the abundance of the detected sequence, clinical or biological evidence, and the possibility of incidental microorganisms. Sequencing can also help monitor pathogen diversity and identify genetic changes that may affect diagnostic performance. Such surveillance is particularly useful when pathogen variation compromises assays that target a limited number of genomic regions.

For practical sericulture, sequencing is likely to complement rather than replace targeted molecular tests [26]. Routine screening may be better served by validated, affordable assays, while sequencing can be reserved for unexplained outbreaks, epidemiological investigations, or the characterization of emerging variants.

Table 2. Molecular Diagnostic Approaches for Silkworm Disease Surveillance

Method	Principal application	Major advantage	Important limitation
Conventional PCR	Detection of DNA targets	Specific and comparatively accessible	Requires appropriate amplification equipment and post-amplification handling
RT-PCR	Detection of RNA targets after reverse transcription	Suitable for RNA viruses and RNA-based targets	Sensitive to RNA degradation and procedural variation
Quantitative PCR (qPCR)	Target detection and relative or quantitative measurement	Real-time monitoring of amplification	Requires validated standards, controls, and assay efficiency
LAMP	Isothermal detection of selected nucleic acid targets	Potential suitability for field-oriented testing	Assay design and contamination control are critical
Targeted sequencing	Pathogen identification and variant characterization	Provides sequence-level information	Limited to selected regions and dependent on target selection
Metagenomic sequencing	Investigation of mixed or unexpected infections	Broad detection potential	Higher analytical complexity and difficulty distinguishing causative from incidental organisms

Note: The optimal method depends on the pathogen, sample type, diagnostic purpose, available infrastructure, and validation requirements.

6. Molecular Regulation of Silk Quality and Fibre Characteristics

6.1 Fibroin and sericin biosynthesis

Silk production is a specialized biological process involving the synthesis, assembly, and secretion of structural proteins by the silkworm silk gland. Fibroin provides the principal structural framework of the silk fibre, while sericin forms a coating that binds the fibroin filaments. Differences in protein composition, molecular organization, and processing contribute to variation in the characteristics of raw silk.

Fibroin biosynthesis involves the heavy chain, light chain, and associated P25 glycoprotein. Their assembly and secretion depend on coordinated gene expression, protein processing, and the cellular environment of the silk gland. Sericin production involves distinct protein components whose expression varies among glandular regions and developmental stages. The molecular regulation of silk production is more complex than the expression of individual structural genes. Regulatory proteins influence tissue differentiation and the spatial activity of silk-related genes, while nutritional status and developmental physiology determine the resources available for protein synthesis [27]. The specialized organization of the silk gland therefore represents an important target for research into both fibre improvement and stress-related production losses.

6.2 Transcriptional regulation and silk-gland development

Gene expression in the silk gland changes during larval development and in response to physiological conditions. Transcription factors, regulatory sequences, signalling processes, and developmental regulators contribute to the differentiated functions of the posterior and middle silk glands. These mechanisms determine when and where particular silk proteins are synthesized. Transcriptomic comparisons among strains with different cocoon characteristics may identify candidate regulatory factors associated with fibroin or sericin production. However, differences in gene expression can also reflect variation in developmental timing, gland size, nutritional condition, or the proportion of different cell types in a sample.

Studies should therefore standardize the developmental stage and sampling procedure and, where possible, combine transcript measurements with protein quantification and silk-gland histology. Functional experiments can then test whether candidate regulatory genes influence silk production directly. A particularly useful approach is to investigate the relationship between silk-gland molecular profiles and commercial fibre characteristics. These characteristics may include cocoon shell weight, shell ratio, filament length, denier, tensile strength, elongation, and processing performance [29]. Linking molecular measurements to these traits can help identify biomarkers with practical value for breeding.

6.3 Nutritional and physiological regulation of silk production

Silk biosynthesis requires substantial nutritional resources, including amino acids derived from feeding and metabolism. The quality of mulberry leaves, feeding rate, digestion, and nutrient allocation can influence the amount of material available for cocoon formation. Environmental stress and disease may further alter this allocation by increasing maintenance requirements or disrupting normal development.

A strain that possesses favourable silk-protein genes may not express its full production potential when nutrition is inadequate or the rearing environment is unsuitable. Conversely, improved nutrition may enhance cocoon performance without necessarily changing the underlying genotype. Molecular improvement should therefore be evaluated under defined nutritional conditions and, where feasible, across different feeding regimes [30]. Research integrating mulberry leaf composition, silkworm metabolic profiles, silk-gland gene expression, and cocoon traits

could clarify how host-plant quality influences silk biosynthesis. Such studies would be valuable for distinguishing genetic limitations from management-related constraints.

6.4 Molecular approaches to silk quality improvement

Molecular breeding can help identify genetic variants associated with silk quantity and quality, while functional genomics can investigate the mechanisms through which these variants influence production. Potential strategies include marker-assisted selection, genomic selection, and targeted modification of genes or regulatory elements involved in silk biosynthesis. Genetic engineering has also been investigated as a means of modifying silk properties or introducing additional protein components. Recent research reviews describe approaches involving exogenous gene introduction, modification of endogenous silk proteins, regulation of the silk-gland environment, and strategies to enhance yield or mechanical performance. These approaches remain subject to important biological and technical constraints. Altering one silk-protein component may affect secretion, assembly, fibre structure, or larval fitness. Increased expression does not necessarily translate into stronger fibres, and improved mechanical properties may not always correspond to better processing performance or greater commercial value [31]. Future research should therefore evaluate silk improvement using multiple complementary measures. Protein composition, fibre mechanics, cocoon traits, processing behaviour, and the biological fitness of the producing silkworm should all be considered. This integrated approach would support improvements that are both technically meaningful and suitable for commercial production.

7. Genome Editing and Advanced Molecular Biotechnology

7.1 CRISPR/Cas-based approaches for genetic improvement

CRISPR/Cas-based genome editing has expanded the capacity to investigate gene function and modify selected genomic regions in silkworms. Unlike conventional breeding, which recombines existing genetic variation over successive generations, genome editing can be used to investigate the effects of targeted sequence changes. Its applications in silkworm research include functional analysis of development, immunity, silk-protein biosynthesis, and other traits relevant to sericulture. The first priority in applying genome editing to disease resistance is the identification of a well-supported genetic target. Candidate genes should be selected using evidence from genomic association studies, transcriptomics, functional experiments, and knowledge of host–pathogen biology. Editing a gene that merely responds to infection may have little benefit, whereas modifying a gene involved in an essential physiological process could introduce undesirable effects.

Functional validation should assess editing efficiency, unintended genetic changes, target-gene function, developmental stability, reproductive performance, and resistance under controlled pathogen exposure. Where possible, edited lines should be compared with appropriate control lines across multiple generations and environmental conditions. These evaluations are necessary to establish whether an observed improvement is reproducible and biologically meaningful.

Recent reviews describe genome editing as part of a broader silkworm engineering toolkit that includes transgenesis, targeted integration, gene silencing, and controlled expression systems. They also identify challenges involving genetic-background dependence, expression consistency, standardization, and biosafety.

7.2 Transgenic approaches and silk-based biomanufacturing

Transgenic technologies have been investigated for introducing selected genetic elements into silkworms, including genes intended to alter silk characteristics or enable the production of additional proteins. The silk gland is of particular interest because of its capacity for high-level protein synthesis and secretion. This has created opportunities to investigate the silkworm as a biological platform for producing engineered fibres and selected recombinant proteins. However, the success of a transgenic strategy depends on stable integration, appropriate expression, correct protein processing, and the absence of unacceptable effects on larval development and reproduction. The expression of a foreign protein does not guarantee that it will be secreted efficiently or incorporated into silk in a functionally useful form.

For applications involving silk-based materials, the final product must be characterized for protein composition, structural organization, mechanical properties, stability, and processing behaviour. For recombinant proteins intended for specialized applications, purity, biological activity, consistency, and appropriate quality-control procedures are also essential.

These applications illustrate how sericulture biotechnology extends beyond conventional cocoon production. Nevertheless, engineered silkworms should be evaluated against the technical, economic, regulatory, and biosafety requirements of their intended use rather than assumed to be commercially viable solely because a laboratory proof of concept has been achieved.

7.3 Combining molecular breeding with conventional selection

Modern biotechnology is most effective when integrated with established breeding programmes. Conventional selection can maintain and improve complex production traits, while molecular markers and genomic information may increase the efficiency of identifying promising individuals. Functional genetics can clarify the mechanisms behind desirable traits, and genome editing may be appropriate for specific targets where its benefits and risks are sufficiently understood.

An integrated programme should begin by defining breeding objectives. These may include resistance to a particular pathogen, stable larval development, favourable cocoon shell ratio, improved silk quality, and

acceptable reproductive fitness. Researchers can then identify candidate genetic markers, validate their association with the target traits, and incorporate reliable markers into selection decisions.

Multi-trait selection is particularly important because resistance and productivity may not always improve together. Selection indices can combine disease response, survival, cocoon yield, silk quality, and reproductive performance according to their economic importance. The relative contribution of each trait should be established using appropriate phenotypic and genetic information.

The ultimate goal is to develop silkworm strains that are biologically robust and commercially useful. A successful molecular breeding strategy should reduce production risks while maintaining the qualities that make silk valuable to farmers, processors, and consumers.

8. Multi-Omics Integration and Bioinformatics in Sericulture

8.1 Integrating genomic and transcriptomic information

Genomics identifies genetic variation, while transcriptomics characterizes patterns of gene activity under particular biological conditions. Combining these approaches can help determine whether genetic variants are associated with changes in gene expression and whether those changes correspond to disease resistance or silk-related traits. For example, a genomic region associated with resistance could be examined alongside transcriptomic data from infected and uninfected larvae. This would help prioritize candidate genes within the region and establish whether their expression differs under pathogen challenge. However, an expression difference does not by itself identify the causal variant, and functional experiments remain necessary to confirm the proposed mechanism.

Bioinformatics is essential for processing and interpreting these datasets. Sequence alignment, variant calling, differential-expression analysis, pathway enrichment, and network analysis can help organize large datasets into testable biological hypotheses. The quality of these analyses depends on suitable reference genomes, accurate annotation, reproducible pipelines, and appropriate statistical methods. Public genomic resources and databases can also improve research efficiency by providing reference sequences, gene annotations, and previously characterized datasets. Their use should be accompanied by clear reporting of database versions, analytical parameters, and data provenance to support reproducibility.

8.2 Proteomics and metabolomics

Proteomics investigates the proteins present in a biological sample, including changes in abundance, processing, and modification. In silkworm disease research, it may help identify proteins involved in immunity, cellular stress, and pathogen interactions. In silk research, it can be used to characterize fibroin, sericin, and associated proteins that influence fibre composition.

Metabolomics provides complementary information about small molecules associated with nutrition, energy metabolism, stress responses, and tissue function. Changes in amino acids, sugars, organic acids, and other metabolites may help explain differences in larval growth or silk production among strains [32]. The integration of these data with genomic and transcriptomic information can reveal where regulatory changes are associated with measurable physiological effects. For example, a change in the expression of a metabolic gene could be evaluated against the abundance of its related metabolites and the resulting effect on larval development. Such evidence is more informative than relying on any single molecular dataset. However, multi-omics integration introduces challenges involving differences in data scale, sample requirements, statistical power, and analytical complexity. Studies should be designed around clearly defined biological questions, with adequate replication and validation rather than collecting large numbers of molecular measurements without a coherent analytical framework.

8.3 Epigenetics and non-coding RNA regulation

Epigenetic mechanisms can influence gene activity without requiring changes to the underlying DNA sequence. DNA methylation, histone modifications, chromatin organization, and non-coding RNAs are potential contributors to the regulation of development, stress responses, and other biological processes in silkworms.

MicroRNAs and other non-coding RNAs are of particular interest because they can regulate gene expression at the post-transcriptional level. Their activity may influence immune responses, developmental processes, and cellular regulation. Nevertheless, the identification of a non-coding RNA that changes during infection does not establish its target or functional contribution. Target validation and appropriate functional experiments are required. Epigenetic research may also help investigate differences among silkworm strains exposed to contrasting rearing conditions. However, environmental effects observed in one generation should not be assumed to represent stable inherited adaptation. Multigenerational experiments are necessary to establish whether a molecular change persists and whether it affects economically relevant traits.

These approaches may eventually complement conventional genetics by explaining some forms of regulatory variation that are not readily captured by DNA sequence markers alone. Their practical contribution to breeding will depend on the reproducibility of the findings and the availability of reliable methods for assessing the relevant traits.

8.4 Artificial intelligence and predictive molecular breeding

Artificial intelligence and machine-learning approaches can help analyse complex genomic, transcriptomic, phenotypic, and environmental datasets. Potential applications include predicting disease susceptibility,

prioritizing candidate genes, estimating breeding values, and identifying relationships among molecular profiles and cocoon characteristics.

Predictive models require representative training datasets, reliable labels, appropriate validation, and careful management of population structure. A model trained on a small number of strains or a single rearing environment may perform poorly when applied to other populations or conditions. Independent validation and transparent reporting of model performance are therefore essential. Machine-learning models should support, rather than replace, biological reasoning and experimental validation. Predictions of disease resistance or silk quality must ultimately be tested using appropriate phenotypic and molecular methods. In practical breeding programmes, model performance should also be evaluated against simpler alternatives to determine whether the additional computational complexity provides a meaningful advantage.

9. Sustainable Sericulture Through Molecularly Informed Disease Management

Molecular biotechnology can contribute to sustainability by improving the accuracy of disease diagnosis, supporting the selection of resistant strains, and reducing avoidable production losses. However, the sustainability of a sericulture system also depends on host-plant cultivation, water and nutrient use, sanitation, waste management, energy consumption, and the economic feasibility of proposed interventions. Early disease detection can help prevent infected material from entering or spreading through rearing systems. Molecular screening of breeding stock may be especially valuable for pathogens capable of persisting or spreading across generations. Such approaches should be combined with appropriate egg screening, sanitation, isolation of affected material, and established biosecurity practices.

Genetically improved silkworm strains may reduce disease-associated mortality and stabilize cocoon production. Nevertheless, resistant strains should be assessed for their broader biological performance, including growth, fertility, developmental timing, and silk quality. Dependence on a narrow genetic base may also introduce vulnerabilities if pathogen populations change or if the selected strain performs poorly under a different environment. Mulberry cultivation is equally important. Suitable cultivars, efficient irrigation, balanced nutrient management, and responsible crop protection can improve host-plant performance while reducing unnecessary resource use. Molecular characterization may help identify desirable plant traits, but field trials remain necessary to confirm that these traits translate into stable leaf production and suitable nutritional quality.

A sustainability assessment should consider the entire production chain. Useful indicators include larval survival, cocoon yield, silk quality, disease incidence, water consumption, nutrient-use efficiency, pesticide dependence, energy use, waste generation, and production costs. This broader evaluation would help determine whether molecularly informed interventions provide genuine environmental and economic benefits.

Table 3. Integration of Molecular Biotechnology with Sustainable Sericulture

Intervention	Primary objective	Potential sustainability benefit	Main implementation consideration
Molecular pathogen diagnostics	Early and accurate infection detection	Reduced disease spread and avoidable losses	Assay validation and testing infrastructure
Disease-resistant breeding	Improve host resistance	More stable cocoon production	Resistance must be balanced with productivity and fitness
Marker-assisted selection	Improve breeding efficiency	More targeted use of breeding resources	Reliable marker–trait associations
Genome editing	Investigate or modify selected genetic targets	Potential for targeted trait improvement	Functional validation, biosafety, and regulatory requirements
Silk-protein optimization	Improve fibre composition or performance	Potential improvement in material quality and value	Protein processing, fibre testing, and commercial validation
Integrated mulberry management	Maintain leaf yield and quality	Improved resource-use efficiency	Local soil, water, and climate conditions
Molecular surveillance of breeding stock	Detect pathogens before dissemination	Reduced risk of recurring or inherited infection	Sampling quality and reliable diagnostic thresholds
Multi-omics and predictive modelling	Integrate complex biological information	More informed selection and management	Data quality, expertise, and independent validation

Note: The benefits described are potential outcomes. Their magnitude depends on biological effectiveness, management conditions, implementation costs, and validation at the production scale.

10. Research Gaps and Future Directions

Despite substantial advances in molecular biotechnology, several challenges continue to limit the translation of laboratory findings into commercially useful sericulture improvements. A major research gap concerns the distinction between molecular responses to infection and mechanisms that genuinely confer resistance. Transcriptomic and proteomic studies frequently identify genes whose expression changes during infection, but these changes may reflect successful defence, pathogen manipulation, or tissue damage. Future studies should

combine gene-expression profiling with functional validation, pathogen-load measurements, and production-related traits. This would help identify candidate genes that have reproducible and biologically meaningful effects. A second priority is the development of silkworm strains that combine disease resistance with high productivity. Resistance against one pathogen does not necessarily confer resistance against another, and improvements in immunity may sometimes be accompanied by changes in growth, development, or reproduction. Breeding programmes should therefore adopt multi-trait selection strategies and evaluate promising strains against multiple pathogens where appropriate. Long-term assessment across different genetic backgrounds and rearing conditions will be essential. A third gap concerns the limited integration of molecular diagnostics with routine disease surveillance. Highly sensitive laboratory methods can improve pathogen identification, but their benefits depend on sampling protocols, turnaround time, affordability, and the capacity to respond to positive results. Future work should develop validated diagnostic workflows that connect early detection with practical decisions about isolation, sanitation, breeding-stock screening, and disease prevention. The improvement of silk quality also requires a more integrated research approach. Many studies focus on silk-protein gene expression or individual fibre characteristics without fully accounting for nutritional conditions, developmental timing, silk-gland physiology, and the genetic background of the silkworm. Research combining molecular profiles with cocoon shell ratio, filament length, fibre strength, elongation, and processing performance could provide a stronger basis for breeding decisions.

Emerging technologies such as genome editing, epigenomics, and multi-omics offer further opportunities, but their use should be guided by clearly defined biological questions. Genome editing requires careful evaluation of unintended effects, developmental stability, reproductive fitness, and regulatory considerations. Epigenetic studies need to distinguish temporary responses from persistent and heritable effects. Multi-omics research should prioritize integrated experimental designs rather than simply increasing the volume of molecular data. Finally, the economic feasibility and environmental consequences of molecular interventions require greater attention. Advanced sequencing and breeding technologies may be inaccessible to some small-scale production systems unless supported by shared facilities, diagnostic networks, extension services, and collaborative breeding programmes. Research should compare their performance with established alternatives and determine which interventions deliver the greatest benefit relative to cost.

Table 4. Priority Research Directions for Molecular Improvement of Sericulture

Research area	Major unresolved question	Recommended approach	Expected contribution
Disease-resistance genetics	Which genes directly contribute to resistance?	Functional validation and pathogen-challenge experiments	Reliable resistance-associated targets
Host–pathogen interactions	How do pathogens alter host defence pathways?	Integrated molecular and pathological analysis	Improved understanding of infection mechanisms
Molecular diagnostics	How can early detection be made more accessible?	Validation of rapid, field-oriented diagnostic assays	Improved disease surveillance
Silk quality genetics	Which molecular changes predict useful fibre characteristics?	Combine silk-gland profiling with fibre phenotyping	Better-informed quality improvement
Multi-trait breeding	Can resistance be improved without reducing productivity?	Genomic and phenotypic selection across multiple traits	More balanced silkworm improvement
Genome editing	Can targeted changes produce stable and safe benefits?	Functional testing and multigenerational evaluation	Evidence-based assessment of edited strains
Multi-omics integration	Which molecular signatures predict actual performance?	Joint analysis of genomic, transcriptomic, proteomic, and phenotypic data	Improved biomarker identification
Sustainable implementation	Which technologies are economically feasible for producers?	Field trials, cost analysis, and environmental assessment	Practical and scalable sericulture innovations

11. CONCLUSION

Molecular biotechnology has expanded the opportunities for improving silkworm disease resistance, silk quality, and the sustainability of sericulture. Genomics and transcriptomics can identify candidate genes and regulatory pathways, molecular diagnostics can improve pathogen detection, and functional genetics can clarify the biological mechanisms underlying resistance and silk production. Marker-assisted selection, genomic selection, RNA interference, and genome editing provide complementary approaches for investigating and improving economically important traits. Nevertheless, the value of these technologies depends on their ability to produce

measurable and reproducible improvements. Molecular responses must be distinguished from functional resistance, and improvements in individual traits must be evaluated alongside larval development, reproductive performance, cocoon yield, and fibre quality. Similarly, diagnostic technologies must be integrated with effective disease-prevention practices, while advanced breeding approaches require validation across relevant genetic backgrounds and production environments. The most promising direction is an integrated framework that connects molecular discovery with conventional breeding, validated diagnostics, silk-gland biology, and sustainable rearing management. Such a framework can help identify silkworm strains that combine disease resistance with productive performance and desirable fibre characteristics. By linking molecular evidence to practical production outcomes, future sericulture research can strengthen disease management, improve the reliability of silk production, and support the long-term economic and environmental sustainability of the industry.

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