**CRISPR and Beyond: A Review of Genome Editing Technologies Transforming Silkworm (*Bombyx mori*) and Mulberry (*Morus* spp.) Research**

**Abstract**

The integration of genome editing technologies, notably CRISPR/Cas systems, has transformed sericulture by allowing for precise and efficient genetic alterations in both *Bombyx mori* and *Morus* spp. Traditional breeding methods have played an important role in silkworm and mulberry advancement, although they are restricted in time, precision, and phenotypic reliance. Recent advances in genome editing have allowed for focused manipulations to improve silk quality, disease resistance, stress tolerance, and biomass output. This study examines the evolution and deployment of important genome editing tools such as ZFNs, TALENs, and CRISPR/Cas variants, focusing on their transformational impact in silkworm strain creation and mulberry feature improvement. It also investigates the use of many omics platforms (genomics, transcriptomics, proteomics, and metabolomics) to drive precision editing and improve functional knowledge. Furthermore, the essay examines the technological challenges, regulatory frameworks, ethical issues, and biosafety problems that come with using altered organisms in sericulture. Looking ahead, developments like multiplex editing, DNA-free editing, and epigenome manipulation provide exciting opportunities for developing a robust, sustainable, and commercially viable sericulture sector.

**Keywords**: CRISPR, Genome editing, Mulberry, Omics, Silkworm.

**1. Introduction**

Sericulture, the practice of silk production through the cultivation of mulberry trees (*Morus* spp.) and silkworm rearing (*Bombyx mori*), has long been integral to the agricultural economies of Asia and certain regions of Europe (Kumar & Sharma, 2024). However, the industry faces mounting challenges, including climate change, pest and disease outbreaks, limited genetic diversity, and the demand for superior quality silk and stress-resistant mulberry varieties (Subramanya Sai Teja & Singh, 2024). Traditional breeding techniques, such as hybridisation, mutation breeding, and selection, have successfully yielded productive silkworm strains and high-yielding mulberry varieties (N. M. Anusha & Vijayan, 2023). Although these strategies have been successful, they are time-consuming, less exact, and strongly reliant on phenotypic selection, rendering them ineffective for meeting the changing needs of modern sericulture.

The introduction of genome editing techniques, particularly CRISPR/Cas9, has altered plant and animal biotechnology by enabling accurate, efficient, and scalable genome alterations (Javaid et al., 2022). These methods allow for targeted gene knockouts, insertions, and even single-nucleotide modifications, giving a strong platform to overcome the limits of traditional breeding. Genome editing in *Bombyx mori* has improved the functional study of silk protein genes, increased disease resistance, and made it easier to create transgenic lines for medicinal protein synthesis (Ijaz & Ul Haq, 2020). Concurrently, the use of these methods in *Morus* spp. is gaining popularity, with potential applications in stress tolerance, nutritional enhancement, and biomass improvement (Sarkar, Mogili, & Sivaprasad, 2017). The silk they produce is not only a luxurious fabric but also a symbol of elegance and craftsmanship. With advancements in biotechnology and genetic engineering, scientists have embarked on a journey to enhance the silk yield and quality of these remarkable insects (Pavithra et al., 2024; Mondal et al., 2007). This review article will look at the most recent advances in genome editing technologies and how they might be used to improve silkworms and mulberry trees. It goes over the existing technologies, successful case studies, technological hurdles, integration with omics techniques, regulatory issues, and future prospects for creating a more sustainable and productive sericulture sector.

**2. Genome Editing Tools: A Brief Overview**

Advances in genome editing tools have transformed the science of functional genomics and expedited trait improvement in both plants and animals(Zhang, Zhang, Lang, Botella, & Zhu, 2017). These techniques enable researchers to do very specific and efficient targeted genetic changes, such as gene knockouts, insertions, or nucleotide substitutions. The most common genome editing platforms are Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein) system (Shamshirgaran et al., 2022).

1. **Zinc Finger Nucleases (ZFNs)**

ZFNs are designed proteins that have both a DNA-binding zinc finger domain and a FokI nuclease domain. Each zinc finger attaches to a unique nucleotide triplet, and numerous fingers can be combined to identify larger DNA sequences(Chivukula et al. 2025). Once attached, the FokI domain causes a double-stranded break (DSB) at the target site. However, ZFNs are costly, technically difficult to construct, and prone to off-target effects, limiting their widespread application.

1. **Transcription Activator-Like Effector Nucleases (TALENs)**

TALENs work similarly to ZFNs, except they employ customised TALE proteins generated from Xanthomonas bacteria to detect particular DNA sequences. Like ZFNs, TALENs rely on FokI to generate DSBs(Sun, 2013). While TALENs are more specific and easier to develop than ZFNs, their enormous size complicates delivery into plant cells or insect eggs. They have been employed in genome editing applications in *Bombyx mori*, notably for preliminary proof-of-concept research (Tsubota et al.,2017).

1. **CRISPR/Cas Systems**

The CRISPR/Cas system, particularly CRISPR/Cas9 from Streptococcus pyogenes, has become the most widely used genome editing tool because to its simplicity, cost-effectiveness, and adaptability. A single-guide RNA (sgRNA) directs the Cas9 nuclease to a specific genomic region, where it introduces a DSB(Xue & Greene, 2021). The cell's repair machinery subsequently repairs the break via non-homologous end joining (NHEJ) or homology-directed repair (HDR), which allows for gene disruption or precise alteration.

1. **Emerging CRISPR Variants:**
2. Cas12a (Cpf1): Produces staggered cuts and needs a distinct PAM sequence, allowing for more targeting versatility(Paul & Montoya, 2020).
3. Base Editing: Enables accurate single-nucleotide modifications without generating DSBs(Molla & Yang, 2019).
4. Prime editing is a flexible approach for accurately inserting or replacing DNA segments(Kantor, McClements, & MacLaren, 2020).

The comparison of these gene editing tools is simplified and given as table below as Table1.

**Table1: Comparison of gene editing tools**

| **Feature** | **ZFNs** | **TALENs** | **CRISPR/Cas9** |
| --- | --- | --- | --- |
| Target Design | Complex | Moderate | Simple |
| Efficiency | Moderate | High | Very High |
| Cost | High | Moderate | Low |
| Off-target Effects | Moderate to High | Low | Low to Moderate |
| Delivery Ease | Moderate | Difficult (large) | Easy |

CRISPR/Cas9 has emerged as the primary genome editing platform in both *Bombyx mori* and *Morus* spp., owing to its simplicity of use, high efficiency, and flexibility to many biological systems(Ma, Smagghe, & Xia, 2019). As the tools advance, they become more precise, allowing for multiplex gene editing and DNA-free genome alterations, which are critical for regulatory approval.

**3. Genome Editing in *Bombyx mori:***

The domesticated silkworm (*Bombyx mori*) is an important species in sericulture and a significant model organism for genetic, developmental, and biotechnological research(Meng, Zhu, & Chen, 2017). The availability of a completely sequenced genome, short life cycle, and simplicity of embryo manipulation make B. mori an attractive target for genome editing studies. In recent years, targeted genome editing, notably CRISPR/Cas9, has allowed the generation of new silkworm strains with improved characteristics, disease resistance, and silk output (Wang et al., 2023).

Trait Improvement through Gene Knockout and Knock-In *B.mori's* genome has been successfully edited to change genes related in color, growth, reproduction, and silk protein synthesis:

1. Silk Quality and Structure: By editing genes such as fibH (fibroin heavy chain), sericin, and BmLP3, the strength, elasticity, and lustre of silk fibres may be altered(Kimoto et al., 2015).
2. Colour Variation: Knocking out the yellow or vermilion genes altered larval or ocular pigmentation, which may be used as identifiers and to create consumer-preferred silk hues (Xiong et al., 2017).
3. Lepidopteran Pest Models: The knockout of certain chemosensory and developmental genes has increased our understanding of insect physiology, which is important for pest control.

**Disease Resistance and Immunity Enhancement**

Silkworms are very sensitive to viral infections such as *Bombyx mori* nucleopolyhedrovirus (BmNPV), which can result in severe economic losses(Tayal & Chauhan, 2017). Genome editing has enabled new strategies:

* Antiviral Resistance: Knocking down certain BmNPV entry receptors or altering immune genes (e.g., BmRelish, BmDicer2) has resulted in the generation of resistant silkworm lines (Xia et al., 2025).
* RNAi Enhancement: CRISPR editing of genes involved in RNA interference pathways has improved natural silkworm resistance to viral infection (Isobe et al., 2004).

**Recombinant Protein Production**

Transgenic silkworms have been developed to manufacture useful bioactive proteins in their silk glands, transforming them into bioreactors.

* Genes encoding human collagen, antimicrobial peptides, and vaccine candidates have been introduced into silk gland-specific loci by CRISPR-mediated knock-in.
* Spider Silk Proteins: High-performance silk proteins from spiders were expressed in B. mori, resulting in stronger, lighter fibres with industrial applications(Spiess, Lammel, & Scheibel, 2010).

**Technical Aspects and Delivery Methods**

* Embryo microinjection is the most popular way of delivering Cas9 mRNA, protein, and sgRNA to early-stage embryos (Joy & Gopinathan, 1994).
* Promoter Selection: The use of tissue-specific (e.g., silk gland) or inducible promoters enables exact temporal and spatial gene expression (Dong et al., 2019).
* Transgenic Systems: The combination of fluorescence reporters and selectable markers allows for easier screening of altered lines (Imamura et al., 2003).

**4. Genome Editing in *Morus spp.* (Mulberry):**

Mulberry (*Morus* spp.) is the sole food supply for the domesticated silkworm (*Bombyx mori*), and so is essential to sericulture (Sarker et al., 1995). Improved mulberry leaf quality, stress resilience, and biomass output have a direct impact on silkworm health and cocoon production (Saini et al., 2023). Traditional mulberry breeding has obstacles such as extended generation times, heterozygosity, and poor seed set in certain elite cultivars (Vijayan et al., 2018). In this context, genome editing technologies, notably CRISPR/Cas systems, provide an effective approach for precise trait change.

**Target Traits for Improvement**

Mulberry genome editing is still in its early stages when compared to silkworm, but it has enormous promise for boosting many critical traits:

1. Drought and salinity Tolerance: Using stress-responsive genes like DREB (dehydration-responsive element-binding) and NAC transcription factors to promote mulberry survival and development in dry and saline environments (Liu et al., 2015).
2. Insect and Disease Resistance involves editing susceptibility genes or improving defence-related pathways to protect mulberry from leaf spot, powdery mildew, and nematodes (Kumari, 2014).
3. Biomass and Leaf Yield: Enhancing vegetative development by modifying growth regulator genes such as GA20ox (gibberellin biosynthesis) or auxin-related genes (Li et al., 2019).
4. Nutritional and phytochemical enhancement includes targeting pathways such as flavonoid, alkaloid, or latex biosynthesis to improve leaf palatability, nutraceutical value, and bioactive component production (Omidiran et al., 2012).

Genetic engineering strategies for mulberry improvement given above are simplified and given as an image in Figure 1.

**Figure 1: Genetic Improvement Strategies for Mulberry**

**Progress and Case Studies**

While published genome editing experiments in mulberry are sparse, similar work in woody plants such as poplar, apple, and citrus provides significant evidence of feasibility:

* CRISPR/Cas9 applications in woody perennials have resulted in disease resistance and growth control, *indica*ting that mulberry may be genetically altered utilising identical tissue culture and transformation procedures (Sarkar et al., 2018).
* Transgenic mulberry research using overexpression and RNAi set the framework for CRISPR intervention by finding functional gene targets (Dhanyalakshmi et al., 2021).
* Several recent Indian and Chinese studies have claimed preliminary success in employing CRISPR/Cas9 to knock off certain transcription factors in *Morus* alba callus or explants.

**Technical Challenges in Mulberry Genome Editing**

* Mulberry's resistance to in vitro regeneration and low transformation efficiency, particularly in elite cultivars, pose significant challenges to genome editing (Sarkar et al., 2022).
* Mulberry's highly heterozygous genome and inadequate reference sequence make it challenging to build sgRNA and forecast off-target events.
* Delivery of Editing Constructs
* The most frequent method is agrobacterium-mediated transformation, which is restricted by genotype sensitivity (Mo et al., 2024).
* DNA-free approaches (e.g., RNP delivery) are being investigated for non-transgenic modifications.
* Chimerism and Somaclonal Variation: Like other woody perennials, regenerants may have a mix of edited and non-edited cells, necessitating many cycles of selection (Datta, 2009).

**Future Outlook for Mulberry Genome Editing**

With the availability of draft genome sequences and better tissue culture techniques, genome editing in *Morus* spp. is likely to grow quickly (Jain et al., 2022). Integration with transcriptome data will allow for the discovery of key gene targets, while advancements in transformation systems (such as the use of growth regulators and somatic embryogenesis) will increase editing efficiency. The creation of gene-edited, non-transgenic mulberry plants might help reduce regulatory hurdles and increase public acceptance.

**5. Integration with Omics Technologies:**

The entire potential of genome editing in *Bombyx mori* and *Morus* spp. is considerably increased by combining it with omics technologies such as genomics, transcriptomics, proteomics, and metabolomics (Fan, Andoh, & Chen, 2023). These technologies enable accurate identification of gene targets, functional validation of altered features, and a better understanding of physiological and biochemical alterations caused by genome editing (Liu et al., 2024).

**Genomics and Genome Annotation**

Whole-genome sequencing of *Bombyx mori* and draft assemblies of several *Morus* species give the genetic blueprints for creating guide RNAs (sgRNAs) for CRISPR/Cas9 editing (Ma et al., 2024). Advances in pan-genomics and comparative genomics aid in the identification of gene families involved in silk production (in silkworms) and abiotic stress response (in mulberry) (Lu et al., 2024). Genome resequencing of various cultivars identifies natural variations and QTLs related to features like as silk quality or drought tolerance, allowing for precise editing (Li et al., 2021).

**Proteomics and Functional Validation**

Proteomics can detect changes in silk protein composition (e.g., fibroin, sericin) following gene editing in silkworms (Dai et al., 2017). Proteomics in mulberry revealed changes in enzyme expression related to photosynthesis, secondary metabolism, and stress signalling following genome alteration (Liu et al., 2024). Protein-protein interaction networks can help guide multiplex gene editing techniques by exposing pathway-level connections.

**Metabolomics and Phytochemical Profiling**

Metabolomic profiling aids in the identification of bioactive chemicals in altered mulberry variants, including flavonoids, latex, and alkaloids (Jiang et al., 2023). It is notably beneficial for confirming nutritional improvement or biopesticide features created by genome editing. Metabolomics is utilised in silkworms to study changes in fatty acid profiles, silk precursor metabolites, and immunological responses (Wang et al., 2019).

**Transcriptomics**

RNA-Seq is used to study gene expression patterns in silkworm tissues (such as silk glands and the midgut) and mulberry leaves under various stress or developmental situations (Luan et al., 2018). RNA-Seq is explained simply by the image given in Figure 2. Differential expression profiling after editing can confirm the success of gene knockouts or activation (de la Peña, Lao, & Bautista, 2022). Transcriptomic data can assist in selecting potential genes for editing, particularly transcription factors, hormone-related genes, and defence genes.



**Figure 2: RNA Sequencing**

**Multi-Omics Integration**

The integration of omics data allows for a systems biology approach to genome editing. Gene-to-phenotype linkage: Using genomics and metabolomics to explain complex features like as drought tolerance (Kumar et al., 2021). Editing target prioritisation involves selecting high-impact genes based on co-expression networks and epigenetic data.

Predictive modelling: Using machine learning on multi-omics datasets, we can anticipate the results of gene editing treatments (Bai et al., 2024). By combining genome editing and omics technology, researchers may create more focused, efficient, and context-specific editing techniques for *Bombyx mori* and *Morus* spp., thereby speeding up breeding operations and improving trait accuracy in sericulture (Ma, Smagghe, & Xia, 2019).

**6. Regulatory, Ethical, and Biosafety Considerations:**

The use of genome editing technologies in agriculture and biotechnology, particularly in economically significant creatures like as *Bombyx mori* and *Morus* spp., poses critical problems concerning legislation, ethics, and environmental safety (Ishii & Araki, 2016). While genome editing offers accuracy and speed, its implementation in the field must adhere to national and international biosafety guidelines to guarantee proper usage (Gene Editing and Agrifood Systems, 2021).

**Regulatory Landscape**

1. **Global Variability**: Regulatory procedures differ around the world. Some nations (e.g., the United States and Japan) exclude genome-edited species with no foreign DNA from GMO regulation (Ishii & Araki, 2017), whilst others (e.g., the European Union and India) apply current GMO rules to all genome-edited organisms.
2. **India's Position**: The Department of Biotechnology (DBT) has developed standards for genome-edited plants, with differences depending on whether foreign DNA is incorporated. Applications for *Morus* spp. may require permission under the Environment Protection Act (1986).
3. **Silkworm-Specific Regulations**: In India and China, the silkworm is regarded as a key bioresource. Any release of altered strains must go through a multi-stage review by sericulture boards and biotechnology agencies (Y. Wang et al., 2023; Zhao et al., 2020).

**Biosafety and Environmental Risk**

1. **Off-Target Mutations**: Despite CRISPR/Cas9's great specificity, unintentional edits can occur. Whole-genome sequencing and phenotypic screening are critical for risk management (X.-H. Zhang et al., 2015).
2. **Gene Flow**: Modified mulberry plants have the ability to hybridize with natural *Morus* species, unwittingly spreading modified features (Andow & Zwahlen, 2006).
3. **Containment Strategies**: Raising silkworms in restricted, sterile circumstances, as well as utilising sterile lines or gene drive blocking techniques, can help to decrease the chance of altered strains escaping into nature (Esvelt et al., 2014).
4. **Monitoring Frameworks**: Long-term monitoring methods should be developed to follow the ecological impact of altered creatures after they have been deployed(Petersen et al., 2021).

**Public Perception and Communication**

1. The general public still has a poor understanding of genome editing. Transparent communication regarding the technology's safety and social advantages is critical to adoption (Petersen et al., 2021).
2. Labelling, traceability, and stakeholder engagement—particularly among sericulture producers and consumers—are crucial for establishing confidence (Lusser et al., 2012).

**Toward Responsible Innovation**

1. **Precautionary principle**: Used mostly in forestry species such as mulberry, owing to long-term ecological interactions.
2. **Ethical Review Committees**: Oversight should encompass multidisciplinary committees of scientists, ethicists, policymakers, and farmers.
3. **DNA-Free Genome Editing**: Using RNP complexes and transient editing methods can help generate non-transgenic plants and animals, decreasing regulatory and public opposition (Y. Zhang et al., 2020).

**7. Conclusion and Future Perspectives:**

Genome editing technologies, notably CRISPR/Cas systems, are transforming sericulture by enabling accurate, efficient, and quick genetic alterations in both *Bombyx mori* (silkworm) and *Morus* spp. (mulberry) (Chen et al., 2021; Kumari et al., 2023). These techniques have already resulted in considerable improvements in silk quality, disease resistance, stress tolerance, and bio-functional characteristics (Bhat et al., 2022). Integrating genome editing with omics technology allows researchers to better comprehend complicated biological networks and hasten the production of elite sericulture cultivars (Bhat et al., 2022).

While genome editing in *Bombyx mori* is currently being used commercially, adoption in *Morus* spp. is still in its early stages, hampered by transformation and regeneration issues (Kumari et al., 2023). Continued advancements in tissue culture, sgRNA design, and delivery technologies, as well as extended reference genomes, will be key to scaling up this technology for mulberry enhancement (L. Zhang et al., 2023).

Looking ahead, various prospective directions stand out.

Multiplex editing is the process of targeting many genes at the same time in order to create multi-trait enhanced silkworms and mulberry plants (M. Wang et al., 2016).

* DNA-Free Editing: The use of ribonucleoproteins (RNPs) to produce non-GMO, regulatory-friendly modified lines (Chen et al., 2021).
* Gene Regulation and Epigenome Editing: New technologies like CRISPRa/i (activation/interference) and base editing will enable fine-tuning of gene expression without changing DNA sequences (Jiang et al., 2021).
* Climate Resilience: By editing genes associated in temperature and drought tolerance, sericulture can better adapt to changing climatic circumstances (L. Zhang et al., 2023).
* Sustainable Bioproducts: Engineered silkworms and mulberry lines can be utilized to make bioplastics, medicines, and functional foods, broadening the economic potential of sericulture (Zhu et al., 2022).

Finally, the success of genome editing in sericulture will be determined not only by technical advancements, but also by open regulatory frameworks, public awareness, and collaborative research among biotechnologists, breeders, and farmers (Bhat et al., 2022). Genome editing has the potential to turn traditional sericulture into a contemporary, climate-resilient, value-added bioindustry by combining scientific innovation with environmental aims.

**Ethical APPROVAL**

1. **Animal Welfare**: Editing B. mori for silk augmentation or immunity poses less ethical problems than vertebrates, although mass rearing and genetic manipulation must still be scrutinised for unforeseen consequences (Ma et al., 2019).
2. **Natural Ecosystems**: Edited mulberry trees or silkworms may interbreed with wild cousins or disrupt predator-prey dynamics, prompting ecological risk assessments (Devos et al., 2022).
3. **Traditional Breeding Displacement**: An overreliance on biotechnology solutions may marginalise indigenous expertise and low-input farming systems (Heinemann et al., 2019).

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**8.Reference:**

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