



वार्षिक प्रतिवेदन ANNUAL REPORT 2024-25



रेशम जैव प्रौद्योगिकी अनुसंधान संस्थान
INSTITUTE FOR SERI-BIOTECHNOLOGICAL RESEARCH
Central Silk Board, Ministry of Textiles
Bangalore - 560035 INDIA

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ANNUAL REPORT 2024-25

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प्राक्कथन

मुझे वर्ष 2024-2025 के लिए केंद्रीय रेशम बोर्ड की रेशम जैव प्रौद्योगिकी अनुसंधान संस्थान (ISBR) की वार्षिक रिपोर्ट प्रस्तुत करते हुए प्रसन्नता हो रही है। यह रिपोर्ट हमारी शोध टीम की अटूट निष्ठा, वैज्ञानिक उत्कृष्टता एवं नवोन्मेषी दृष्टिकोण को दर्शाती है। वन्या तथा शहतूत दोनों क्षेत्रों के उन्नयन के प्रति हमारी प्रतिबद्धता, रेशम उद्योग के लिए उन्नत प्रौद्योगिकियों, सतत समाधानों एवं प्रभावशाली वैज्ञानिक योगदानों के विकास में निरंतर प्रेरणा प्रदान कर रही है।



इस वर्ष हमारी प्रयोगशाला ने कई महत्वपूर्ण शोध उपलब्धियाँ प्राप्त कीं। *Antheraea assamensis* Cypovirus-4 (AaCPV-4) के संपूर्ण जीनोम का सफलतापूर्वक अनुक्रमण एवं प्रकाशन किया गया, जो मुगा रेशमकीट में वायरल रोगों को समझने की दिशा में एक महत्वपूर्ण कदम है। मिलीबग के पर्यावरण-अनुकूल जैविक नियंत्रण हेतु वैक्स-विघटित करने वाले जीवाणुओं की पहचान की गई। ट्रांस्क्रिप्टोम विश्लेषण के माध्यम से CPV संक्रमण के प्रति प्रतिक्रिया में मुगा रेशमकीट के प्रमुख प्रतिरक्षा-संबंधी जीनों एवं नवीन miRNA की पहचान की गई, जो उन्नत रोग प्रबंधन रणनीतियों के विकास में सहायक सिद्ध हो सकती है। इसके अतिरिक्त, पिंग मिलीबग की लार में महत्वपूर्ण प्रोटीनों की पहचान की गई, जो कीट नियंत्रण हेतु संभावित RNAi लक्ष्यों के रूप में उपयोगी हो सकते हैं। साथ ही, चार आनुवंशिक रूप से स्थिर एरी रेशमकीट प्रजनन लाइनों का विकास किया गया तथा महत्वपूर्ण रेशमकीट गुणों से जुड़े आनुवंशिक लोकी की पहचान की गई, जो वान्या रेशमकीट प्रजनन कार्यक्रमों के सुदृढीकरण में योगदान देगी।

इन उपलब्धियों के साथ-साथ, संस्थान द्वारा रेशमकीट एवं शहतूत दोनों के लिए आणविक बारकोड के विकास की पहल की गई। इसके अतिरिक्त, जैव-चिकित्सीय अनुप्रयोगों हेतु प्यूपल तेल आधारित लिपोसोम तथा बैकमिड प्रणालियों के विकास की दिशा में भी प्रयास जारी हैं।

मैं केंद्रीय रेशम बोर्ड के सदस्य सचिव एवं निदेशक (वित्त) का उनके निरंतर सहयोग के लिए हार्दिक आभार व्यक्त करता हूँ तथा अनुसंधान परामर्श समिति का उनके मूल्यवान मार्गदर्शन के लिए धन्यवाद देता हूँ। साथ ही, हमारे वित्तपोषण एजेंसियों एवं सहयोगी संस्थानों के निरंतर समर्थन के लिए भी आभार प्रकट करता हूँ। ISBR के वैज्ञानिकों एवं कर्मचारियों की निष्ठा, परिश्रम एवं योगदान के लिए मैं विशेष रूप से सराहना करता हूँ।

मुझे आशा है कि यह रिपोर्ट आपको उपयोगी, ज्ञानवर्धक तथा अनुसंधान एवं नवाचार के माध्यम से रेशम क्षेत्र को सुदृढ करने के प्रति हमारी प्रतिबद्धता का सटीक प्रतिबिंब प्रतीत होगी।

डॉ. एस. मंदिरा मूर्ति
निदेशक

FOREWORD

I am pleased to present the annual report of the Institute for Seri-Biotechnological Research (ISBR), Central Silk Board, for the year 2024–2025. This report showcases the unwavering dedication, scientific excellence, and innovative mindset of our research team. Our commitment to advancing both the Vanya and mulberry sectors continues to drive our efforts in developing cutting-edge technologies, sustainable solutions, and impactful scientific contributions for the sericulture industry.



During this year, our laboratory achieved several important research milestones. The complete genome of *Antheraea assamensis* Cypovirus-4 (AaCPV-4) was successfully decoded and published, marking a significant step toward understanding viral diseases in the muga silkworm. Wax-degrading bacteria were identified that can be used for eco-friendly biological control of mealybug. Through transcriptome analysis, key immune-related genes and novel miRNAs responding to CPV infection were identified in the muga silkworm, which may aid in developing improved disease management strategies. Furthermore, important salivary proteins in the pink mealybug were identified as potential RNAi targets for pest control. In addition, four genetically stable eri silkworm breeding lines were developed and utilized to identify genetic loci linked to important silkworm traits, which will contribute to the improvement of Vanya silkworm breeding programmes.

Along with these, the institute initiated the development of molecular barcodes for both silkworm and mulberry. Furthermore, efforts are ongoing toward the development of pupal oil-based liposomes and bacmid systems for biomedical applications.

I express my sincere thanks to the Member Secretary and Director (Finance) of the Central Silk Board for their constant support, and to the Research Advisory Committee for their valuable guidance. I also thank our funding agencies and collaborating institutions for their continued support. My heartfelt appreciation goes to the scientists and staff of ISBR for their dedication, hard work, and contributions.

I hope you find this report useful, informative, and a reflection of our commitment to strengthening the sericulture sector through research and innovation.

Dr. S. Manthira Moorthy
Director

VISION, MISSION AND MANDATE

VISION

To become a Centre of Excellence in Seri-biotechnology

MISSION

To achieve excellence in research in frontier areas of modern biology to transform Indian Sericulture Industry into a competitive commercial production base

R & D

To conduct research in the frontier areas of biotechnology to enhance quality & quantity of silk production as well as value addition through non-textile applications.

Collaborate

To collaborate with national and international organizations for strengthening the basic and applied research in Seri-biotechnological aspects.

MANDATE

COMMERCIALIZE

To disseminate and commercialize the tools & technologies among the stakeholders.

HRD

To develop quality human resources in the area of Seri-biotechnology.



अनुसंधान के मुख्य आकर्षण

1. एंथेरिया एसामेंसिस सिपोवायरस-4 (AaCPV-4) के संपूर्ण जीनोम का सफलतापूर्वक अनुक्रमण किया गया।
2. पिंक मिलीबग के पर्यावरण-अनुकूल जैविक नियंत्रण हेतु उपयोगी वैक्स-विघटित करने वाले जीवाणुओं की पहचान की गई।
3. ट्रांस्क्रिप्टोम विश्लेषण के माध्यम से सी पी वी संक्रमण के प्रति प्रतिक्रिया में मूगा रेशमकीट के महत्वपूर्ण प्रतिरक्षा जीनों एवं नए माइक्रो-आर एन ए की पहचान की गई।
4. मिलीबग की लार में महत्वपूर्ण प्रोटीनों की पहचान की गई, जो कीट नियंत्रण के लिए संभावित आर एन ए-आई लक्ष्य हो सकते हैं।
5. चार आनुवंशिक रूप से स्थिर एरी रेशमकीट प्रजनन लाइनों का विकास किया गया तथा शरीर के रंग से जुड़े एस एन पी लोकेस की पहचान की गई।

RESEARCH HIGHLIGHTS

1. Successfully decoded the complete genome of *Antheraea assamensis* Cypovirus-4 (AaCPV-4).
2. Identified wax-degrading bacteria that can be used for eco-friendly biological control of pink mealybug.
3. Conducted transcriptome analysis revealing key immune-related genes and novel miRNAs involved in the muga silkworm response to CPV infection.
4. Characterized pink mealybug saliva and identified critical proteins that serve as promising RNAi targets for sustainable pest management.
5. Developed four genetically stable eri silkworm breeding lines and mapped SNP loci associated with body colour traits.

LIST OF RESEARCH PROJECTS

ON-GOING RESEARCH PROJECTS

Project Code	Project Title	Duration
AITo8o1oMI	Transcriptome Studies for Susceptibility: Transcriptome studies for susceptibility of Muga silkworm (<i>Antheraea assamensis</i> Helfer) to cypovirus	Apr 2022 – Mar 2026
SRPo8o12MNC	Rapid Antigen Test Kit: Development of a Rapid Antigen Test Kit for Diagnosis of Cytoplasmic Polyhedrosis in Vanya Silkworms (<i>Antheraea assamensis</i> , <i>A. mylitta</i> and <i>A. proylei</i>)	Feb 2024 – Jan 2026
SITo8o14 MIC	Molecular Barcodes: Development of molecular barcodes for commercially important silkworm (<i>Bombyx mori</i>) breeds/ hybrids	Mar 2024 – Feb 2026
SITo8o15 MIC	SNP Marker Validation: Validation of SNP markers associated with economic traits in <i>Bombyx mori</i> L.	Mar 2024 – Feb 2026
PRPo8o13MIC	Mulberry Rootstock Mapping: Mapping and Evaluation of Mulberry Rootstock for Root rot (<i>Lasiodiplodia theobromae</i>) Resistance	Mar 2024 – Feb 2027
PRPo8o19MIC	DNA Barcoding: DNA barcoding of Mulberry germplasm and authorized mulberry varieties .	Jan 2025 – Dec 2026
BPSo8o17SNC	Biomedical Liposomes: Development of silkworm pupal phospholipid-based liposome for biomedical applications.	Jan 2025 – Dec 2027
SITo8o18SNC	Recombinant Protein System: Construction and Efficacy Assessment of a BmNPV Derived Bacmid System for Recombinant Protein Production in <i>Bombyx mori</i> Silkworms	Jan 2025 – Dec 2027
SITo8o16SNC	Non-Textile Silk: Cloning and Expression of Multifunctional Recombinant Fibroin Silk proteins for Non-Textile applications.	Jan 2025 – Dec 2027

LIST OF RESEARCH PROJECTS

CONCLUDED RESEARCH PROJECTS

Project Code	Project Title	Duration
ARPo8007MI	Virosis Characterization: Biological and molecular characterization of virosis in Muga silkworm	Mar 2022 – Feb 2025
AIBo8008MI	Eri Breed Development: Development and Evaluation of Eri silkworm (<i>Samia ricini</i> Donovan) breeds/ hybrids with improved productivity .	Mar 2022 – Feb 2025
AI Co8009CN	Mealybug Control: Profiling of lipid, protein and carbohydrate of mulberry mealybug <i>Maconellicoccus hirsutus</i> (Green) for its control	Apr 2022 – Mar 2025

PROGRESS OF ONGOING RESEARCH PROJECTS



1. AITo8o1oMI: Transcriptome studies for susceptibility of Muga silkworm (*Antheraea assamensis* Helfer) to cypovirus

(Collaboration with MESSO, Guwahati; Duration: April 2022-March 2026)

Himanshu Dubey, Rajal Debnath, G. Subrahmanyam, Vikram Kumar², & Abhishek Singh³

²P3 unit of MESSO Rompara, Meghalaya; ³P3 Unit of MESSO Nongpoh, Meghalaya

Objectives:

1. Analysis of miRNA mediated modulation in immunity during cypovirus infection.
2. Validation of differentially expressed genes and miRNAs by qRT-PCR.
3. Identification of suitable target genes for marker development.

Antheraea assamensis, commonly known as the muga silkworm, is an economically important lepidopteran species endemic to the northeastern region of India, renowned for producing the golden-hued muga silk. As it is reared in outdoor conditions and is frequently exposed to various environmental stressors, including pathogenic infections. Among these, viral pathogens such as **Cypovirus** pose a significant threat, leading to high larval mortality and substantial economic losses in sericulture. In this project, transcriptome analysis was conducted to investigate the molecular response of *Antheraea assamensis* to Cypovirus infection. Differential expression analysis revealed **283 genes** in the **midgut** and **184 genes** in the **fat body** showing significant regulation (**Fig. 1**). These differentially expressed genes were primarily enriched in immune and stress response pathways, including **prophenoloxidase**, **serine/threonine-protein kinase**, **glutathione S-transferase (GST)**, **cytochrome P450 (CYP450)**, **trypsin alkaline**, **death-associated inhibitor of apoptosis 1**, **Krüppel-like factors**, and **heat shock proteins**, highlighting the activation of key antiviral and detoxification mechanisms (**Fig. 2**).

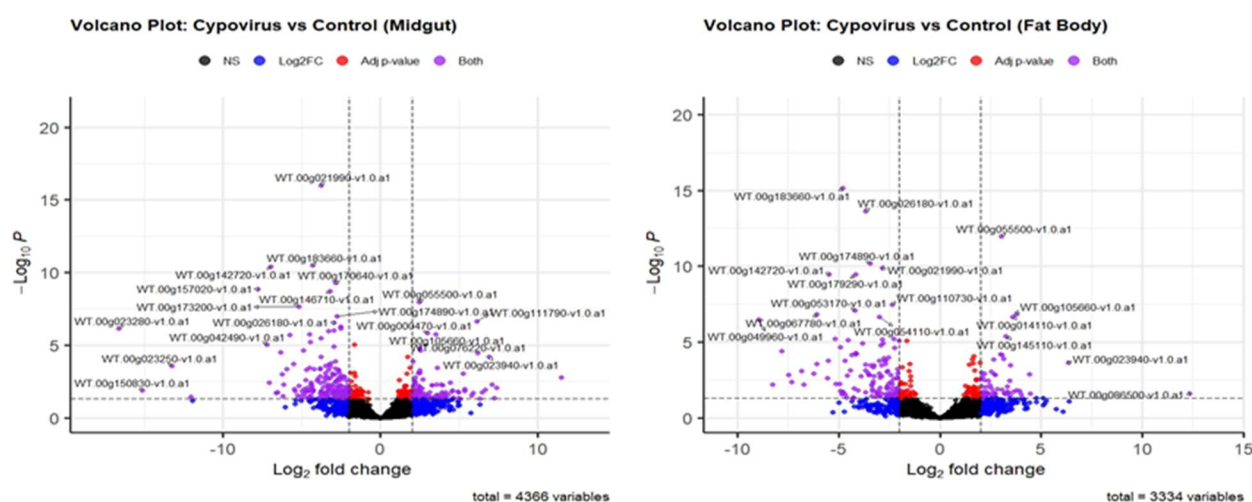


Figure 1. Volcano plots showing differential gene expression between Cypovirus-infected and control larvae in midgut and fat body tissues. The x-axis represents the $\log_2$ fold change in expression, with negative values indicating downregulation and positive values indicating upregulation in response to viral infection. The y-axis represents the $-\log_{10}$ of the adjusted p-value, where higher values indicate greater statistical significance.

In parallel, small RNA sequencing was performed to identify virus-responsive miRNAs. In the **fat body**, **10 significant miRNAs** were identified, among which **9 were novel**, and one showed sequence similarity to *Drosophila* miRNAs. In the **midgut**, **20 miRNAs** were found significant, including **17 novel miRNAs**, **2 matching *Bombyx mori***, and one corresponding to "**tcf-miR-193**"(Fig. 3). Notably, **tcf-miR-193** is associated with the **TCF family of transcription factors**, which are involved in the regulation of gene expression related to development and immunity. These miRNAs may play key post-transcriptional regulatory roles during viral infection, offering new insights into immune modulation in this economically important lepidopteran species

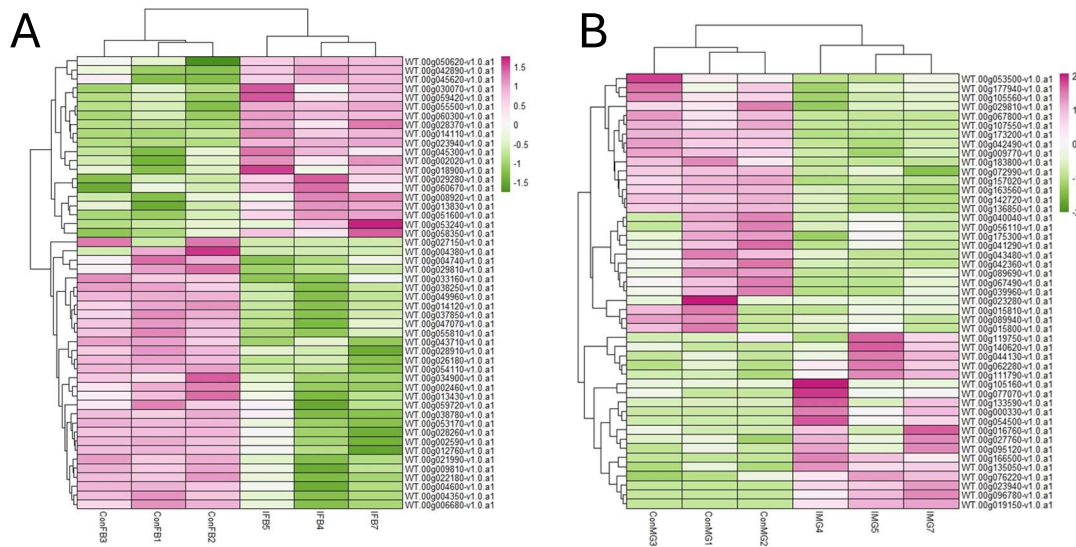


Figure 2. Heatmap representation of differentially expressed genes in (A) midgut and (B) fat body tissues of *A. assamensis* larvae following Cypovirus infection compared to control groups. Each row corresponds to a transcript, while columns represent biological replicates of infected (I) and control (Con) samples. The color scale indicates relative expression levels, with green representing downregulation, magenta representing upregulation, and white representing intermediate expression. Hierarchical clustering of both genes and samples demonstrates distinct expression patterns between infected and control groups

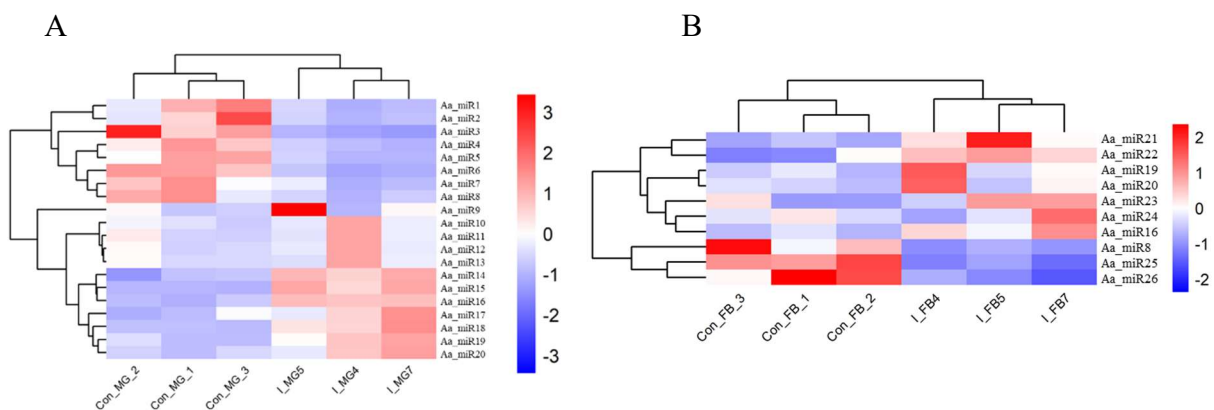


Figure 3. Heatmap of differentially expressed miRNAs in (A) midgut and (B) fat body tissues under different experimental conditions. Rows represent individual miRNAs, while columns correspond to the biological replicates of infected (I) and control (Con) samples of midgut (MG) and fat body (FB) tissues, respectively. Expression levels are displayed using a color gradient, where red indicates upregulation, blue denotes downregulation, and white represents intermediate expression.

2. SRPo8012MNC: Development of a Rapid Antigen Test Kit for Diagnosis of Cytoplasmic Polyhedrosis in Vanya Silkworms (*Antheraea assamensis*, *A. mylitta* and *A. proylei*)

(In collaboration with CMER&TI Lahdoigarh and CTR&TI, Ranchi; Duration: February 2024 -January 2026)

G. Subrahmanyam, Soumya Paul¹, Rajal Debnath, HS. Gadad², Brunda³

¹Pentavalent Biosciences Pvt. Ltd, Bangalore

²CSB-CTR&TI Ranchi; ³CSB-CMER&TI, Lahdoigarh

Objectives:

1. To develop a rapid antigen test kit (RAT) and to optimize the kit for detection of CPV-4 in vanya silkworms.
2. To develop chemical-based disinfectant to control cypovirus-4 infection in rearing fields.

The Muga silkworm is an economically significant, multivoltine, and semi-domesticated insect species, endemic to the North Eastern region of India and recognized as a Geographical Indicator. Since the species is reared outdoors on standing host trees, it is highly susceptible to diseases such as pebrine, flacherie, muscardine, and cytoplasmic polyhedrosis (CPV subtype 4), which together contribute to substantial crop losses. Cytoplasmic polyhedrosis, in particular, is a major threat, commonly occurring during the summer season and causing up to 40% mortality in rearing crops. The infection usually remains unnoticed until the 5th instar, when symptoms appear and larvae die rapidly within 48 hours, severely impacting cocoon yield. Therefore, early detection of CPV infection and the implementation of preventive measures, including field disinfection and timely management strategies, are crucial for sustaining the productivity and economic stability of Muga sericulture in Northeast India.

The CPV-infected muga larvae yielded high-quality RNA suitable for cDNA synthesis and PCR amplification. The polyhedrin gene was amplified and cloned into the pJET1.2/blunt vector and later subcloned into the pET21b expression vector. Colony PCR and sequencing confirmed the correct orientation and integrity of the insert within the recombinant plasmid (**Fig. 4**). Transformation of the recombinant pET21b–polyhedrin construct into *E. coli* BL21 (DE3) cells followed by IPTG induction resulted in high-level expression of the recombinant polyhedrin protein. SDS-PAGE analysis revealed a distinct, sharp band at approximately 29 kDa corresponding to the expected size of the recombinant polyhedrin with a C-terminal His-tag (**Fig. 5**). The recombinant protein was purified through Ni–NTA affinity chromatography, yielding a highly pure antigenic protein suitable for immunization. Mice immunized with the purified recombinant polyhedrin antigen exhibited strong immune responses, as evidenced by high antibody titers. Hybridoma technology was being employed to generate monoclonal antibody producing cell lines specific to CPV polyhedrin.

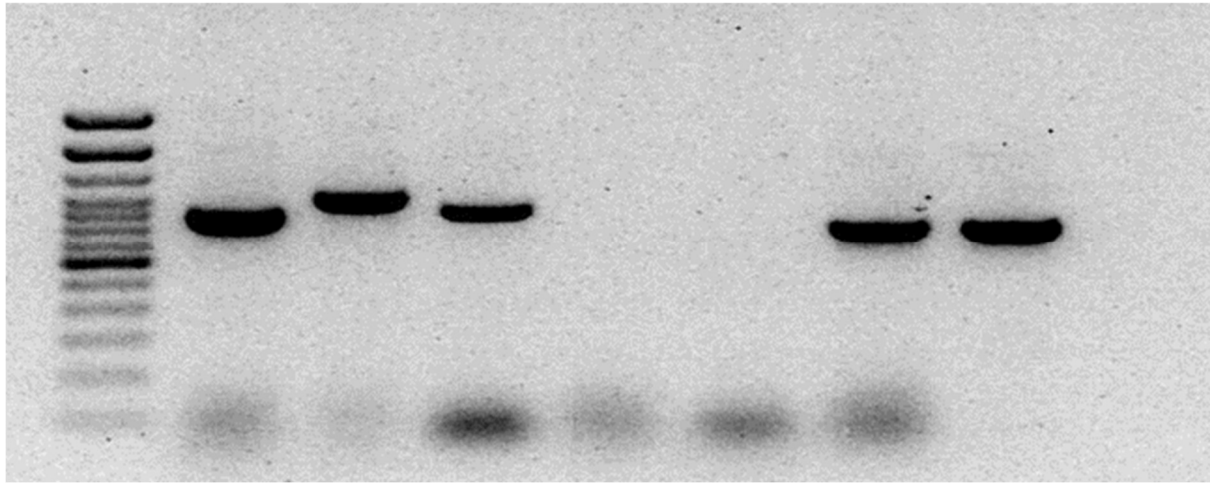


Figure 4: Cloning of viral polH gene. Confirmation of insert through amplifications with Gene specific (pol H) and vector specific primers (T7). Lane1: Ladder (100 bp); Lane2: GSR + VSF (886 bp); Lane3: VSF + VSR (974 bp); Lane4: GSF + VSR (895 bp); Lane5: VSF + GSF; Lane6: GSR + VSR; Lane7: GSF + GSR (764 bp); Lane8: Positive control (polH gene, 764 bp); Lane9: NTC.

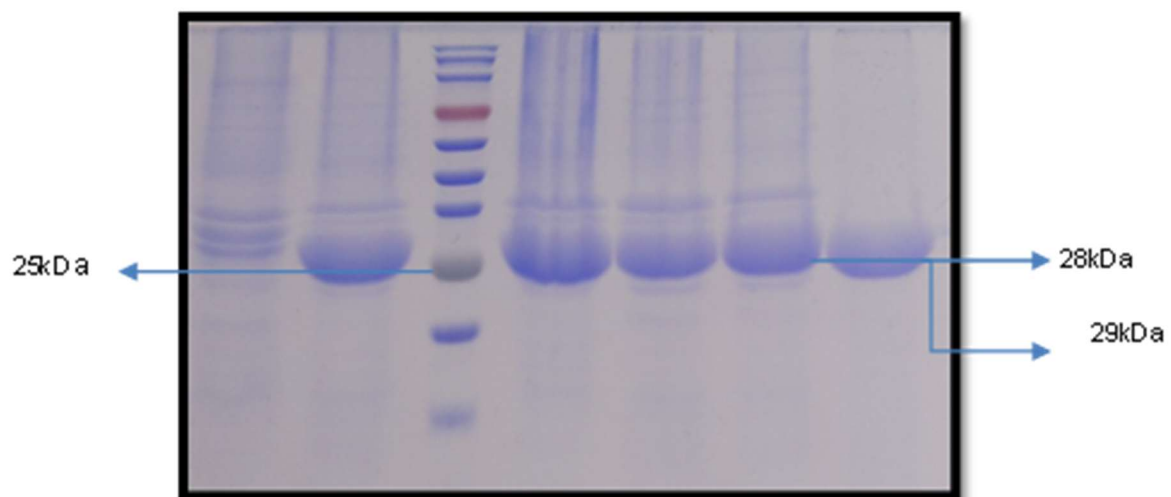


Figure 5: Heterologous expression and purification of polH gene in *E. coli*. Lane 1: Uninduced sample; Lane 2: Induced sample (Pol H – 29 kDa); Lane 3: Ladder (250 kDa); Lanes 4–6: Induced samples; Lane 7: CPV purified sample (Pol H – 28 kDa).

3. SITo8014MIC: Development of molecular barcodes for commercially important silkworm (*Bombyx mori*) breeds/hybrids

(In collaboration with CSR&TI, Mysore; CSGRC, Hosur and P3 Farm, NSSO, Mysore; **Duration:** March 2024 - February 2026)

Himanshu Dubey, Rajal Debnath, G. Lokesh¹, Kusuma L², Tushar Ramachandra Khare³

¹CSGRC, Hosur; ²CSR&TI, Mysore; ³P3 Farm, NSSO, Mysore

Objectives

1. Whole genome sequencing of CSR6, CSR26, CSR27 and SK7 to identify variations for marker development.
2. Development and validation of molecular markers for uniqueness and specificity to the respective breeds.

The absence of a well-established method for parentage or hybrid verification poses a significant challenge in ensuring genetic purity and managing trait deviations in sericulture. Accurate parentage verification is also crucial for protecting breeders' intellectual property and for the reliable identification of the true FC1 × FC2 double hybrid. To address this gap, we analyzed the *Bombyx mori* pangenome datasets and identified thousands of SNP loci (**Fig 6**) with potential for discriminating silkworm accessions, from which initially 22 SNPs were selected for validation using Sanger sequencing. While *in-silico* validation confirmed primer design feasibility, downstream sequencing revealed that only one locus consistently generated clear, high-quality read across all genomes, with the others producing multiple peaks, likely due to genomic duplications or related complexities. Subsequently, we performed a combined analysis of *Bombyx mori* pangenome and GBS datasets to identify loci suitable for SNP-based barcoding of CSR2, CSR6, CSR26, and CSR27, leading to the successful development of barcodes further validated using ddRAD analysis. A low-cost validation assay, such as tetra-primer ARMS-PCR or allele-specific PCR, will now be developed based on these loci. In addition, samples of CSR6, CSR26, CSR27, SH6, NB4D2, and SK7 have been collected and submitted for whole-genome sequencing, creating a foundation for robust, molecular marker-based parentage verification to strengthen breeding programs and ensure genetic purity in the silk industry.

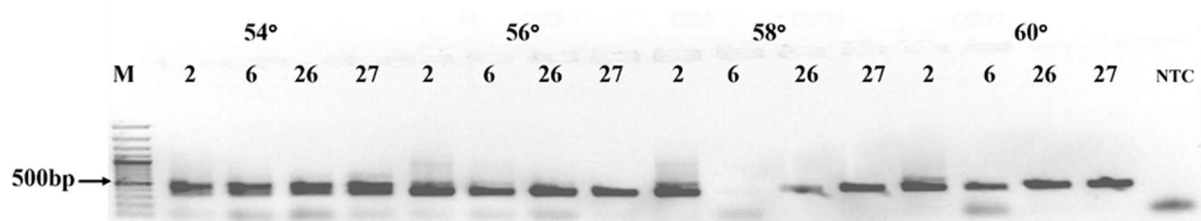


Figure 6: Agarose gel electrophoresis image showing annealing temperature optimization for PCR amplification of selected SNP loci.

4. SITo8015MIC: Validation of SNP markers associated with economic traits in *Bombyx mori* L.

(In collaboration with CSR&TI, Mysore and P3 Farm, NSSO, Mysore; Duration: March 2024 - February 2026)

Himanshu Dubey, Rajal Debnath, Kusuma L¹, N. Chandrakant¹, K. M. Ponnuvel²

¹CSR&TI, Mysore; ²P3 Farm, NSSO, Mysore

Objectives

1. Development of F2 population for validation of SNP markers.
2. Genotyping and association analysis of SNP markers with selected trait.

The present study focuses on the validation of SNPs associated with key economic traits in *Bombyx mori* L., initially identified under the DBT-sponsored project AIT-3628. In the current work, efforts are directed toward developing a segregating population for cocoon weight and shell weight using contrasting parents and testing the association of SNPs in a large population. In this context, segregating population using ten parental combinations prepared at collaborating institute. The GBS data was re-analyzed using STACKs pipeline with updated genome assembly of *Bombyx mori*. Genome-wide association analysis was performed with phenotypic data using PLINK software which revealed suggestive association of SNPs from Chromosome 1, 8, 15, 20, 21 and 25 (**Fig. 7**).

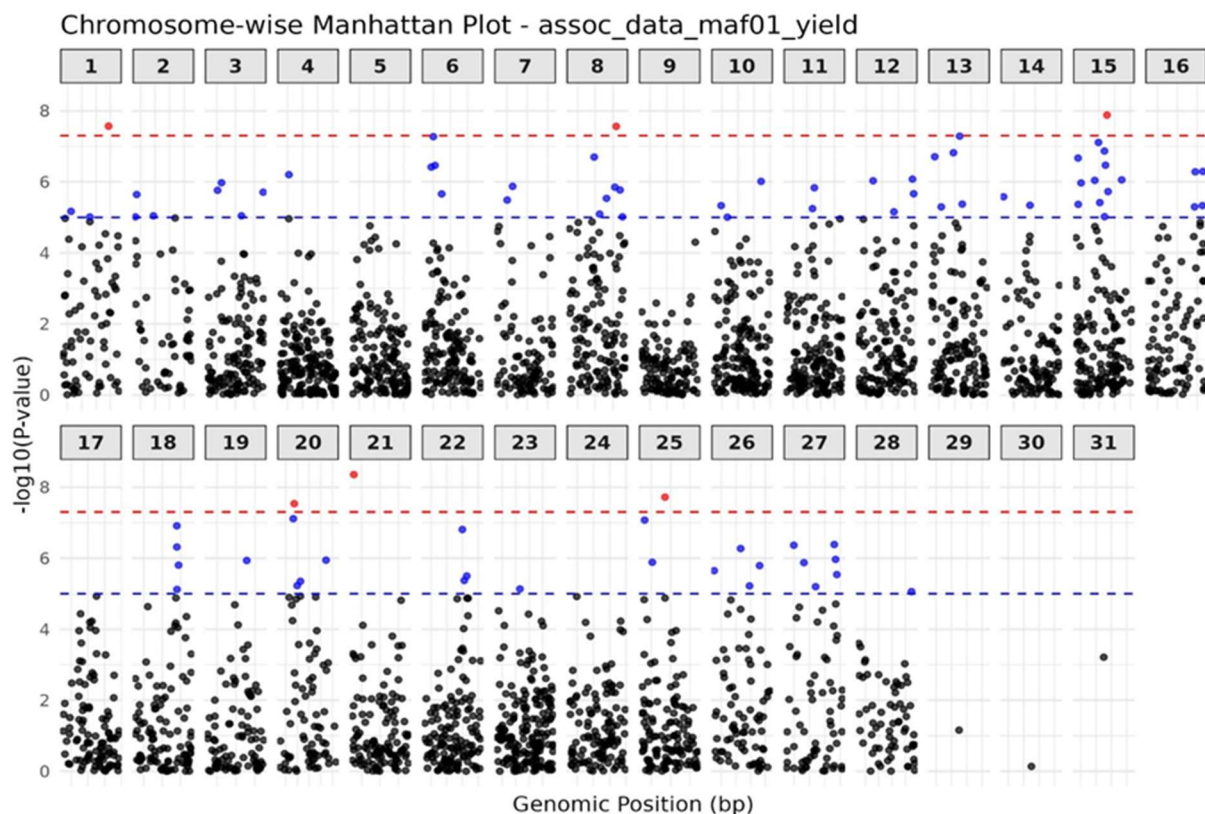


Figure 7: GWAS analysis of yield trait in *B. mori* using reanalyzed GBS data. Red dots represent SNP loci with suggestive association to yield traits.

5. PRPo8013MIC-Mapping and Evaluation of Mulberry Rootstock for Root rot (*Lasiodiplodia theobromae*) Resistance

(In collaboration with CSR&TI, Mysore; Duration: March 2024 - February 2027)

Ramesha A., Pawan Shukla, Himanshu Dubey, Arunakumar G. S.*

*CSR&TI, Mysore

Objectives

1. To identify genomic regions/genes associated with root rot resistance in mapping population
2. To evaluate potential rootstock-scion combination for root rot resistance and scion traits through grafting

Root rot, a significant soil-borne disease, severely impacts mulberry cultivation in Southern India by reducing leaf yield and quality, which are critical for silk production. Traditional breeding methods to develop resistant mulberry varieties are inefficient due to the need to screen vast populations and the influence of environmental factors on disease phenotype screening. Therefore, the use of molecular markers is a more efficient and reliable approach for breeding. The ultimate goal of mulberry breeding is to combine as many desirable traits as possible into a single variety. However, this is difficult with traditional hybridization, which involves only two parents. This project addresses this challenge by employing a grafting approach, which by grafting a high-yielding scion onto a rootstock with a high level of root rot resistance. The project has a two-fold aim: first, to identify the genomic regions or genes responsible for root rot resistance; and second, to develop rootstock-scion combinations for ensuring both disease resistance and good yield.

Bi-parental mapping population developed at CSR&TI, Mysore by crossing *Morus multicaulis* (root rot resistant) with Thailand Male (root rot susceptible genotype) was used for mapping root rot resistance through high throughput genotyping by ddRAD sequencing. In addition, SSR markers for mapping root rot resistance were identified by downloading genome data of parental accessions, *M. multicaulis* and Thailand Male from NCBI. Adapter trimming and removal of low-quality bases followed assembly of data by Spades de-novo program.

Assembled contigs were used for SSR prediction using MISA tool, 150bp up and downstream of the SSR from genome were extracted and their conservation was checked in *M. multicaulis* genome. Conserved up and downstream sequences were selected for primer designing using Primer3, SSRs with motif length of ≥ 50 bp were selected. In-silico PCR was performed to check cross-transferability, number of amplicons and chromosomal distribution. Subsequently, SSR markers were assessed through PCR (Fig. 8).

The root rot resistant mulberry accession, *Morus multicaulis* was used as a rootstock and V1 variety as a scion to generate grafted plants. Bud and cleft grafting approach was taken to establish the grafted plants (**Fig. 9**).

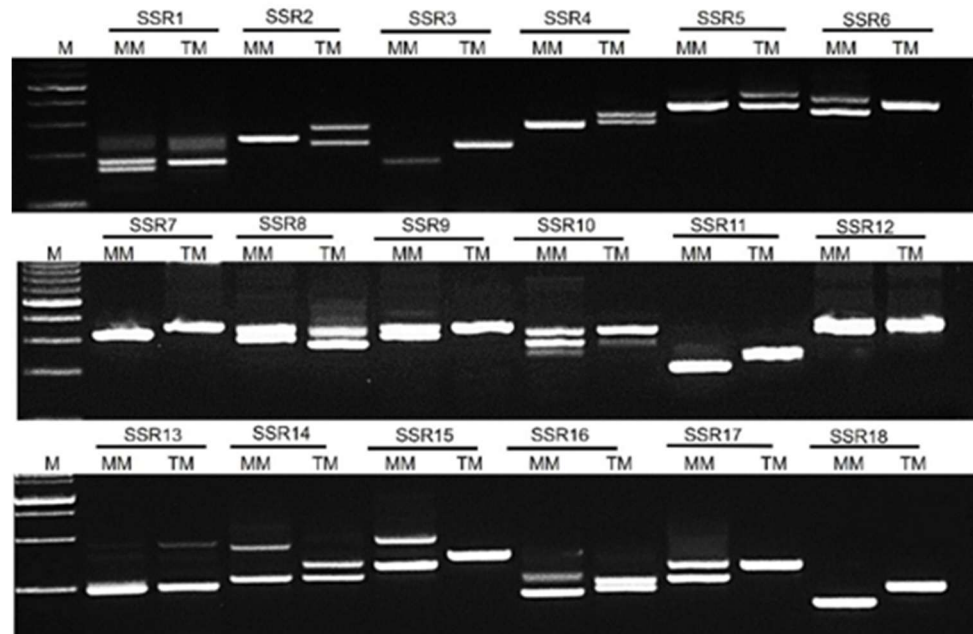


Figure 8: SSR markers identification and analysis: Identified 58 polymorphic SSR markers between *M. multicaulis* and Thailand male



Figure 9: Grafted mulberry plants for root rot resistance were generated using *Morus multicaulis* as a rootstock and V1 as a scion.

6. PRPo8019MIC-DNA barcoding of mulberry (*Morus* spp.) germplasm and authorized mulberry varieties

(In collaboration with CSGRC, Hosur; Duration: January 2025 - December 2026)

Ramesha A, Rajal Debnath, Himanshu Dubey, Raju Mondal*

*CSGRC, Hosur

Objectives

1. To identify core set of mulberry germplasm at species level using DNA barcode
2. To establish genome wide allele profiles for authorized mulberry varieties to enable precise varietal identification, and their protection

Each mulberry species possesses unique traits essential for specific uses, making the comprehensive exploration, conservation, and genetic characterization of mulberry germplasm critically important. The Central Sericultural Germplasm Resources Centre (CSGRC) currently conserves 1,317 diverse mulberry germplasm accessions. Traditional plant taxonomy has significant limitations leading to variability and potential misidentification. These challenges underline the need for molecular techniques like DNA barcoding, which offer more precise and reliable species classification by incorporating genetic data. An essential aspect of this characterization is accurately assigning mulberry accessions to the correct species, which is vital for their optimal use, improvement through breeding program, and elimination of duplicates. The core set and accessions possessing desirable traits such as biotic and abiotic stress tolerance and fruit yielding will be barcoded.

Commercial mulberry varieties are critical for the sericulture industry, and proper management, ownership, and enhancement of their germplasm are vital for the sector's growth. Traditional morphological identification methods are time-consuming, costly, and often unreliable due to phenotypic plasticity. One effective solution to these challenges is the development of DNA-based signatures for mulberry varieties. DNA profiling and barcoding can complement traditional DUS (distinctness, uniformity, and stability) testing, improve accuracy and support varietal protection. In this study, Allele profiling of authorized mulberry varieties will be done by genomic information.

Polymorphic SSR markers identified from *Morus* spp. were used to test the polymorphism in the authorized mulberry varieties collected from CSR&TI, Mysore. The genomic DNA was isolated from authorized varieties and PCR was performed with SSR primers and analyzed in metaphore agarose gel and scored for polymorphic banding pattern (**Fig. 10**).

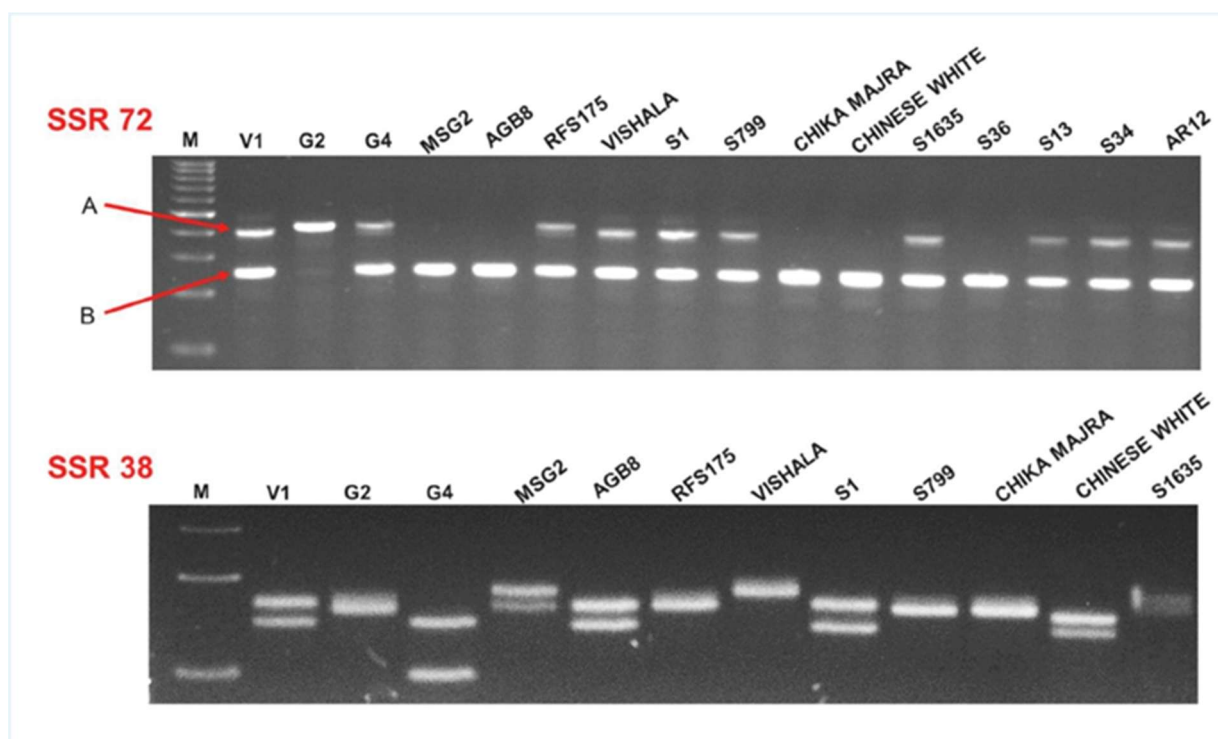


Figure 10: SSR markers analysis in authorized mulberry varieties for allele profiling

7. BPS08017SNC: Development of silkworm pupal phospholipid-based liposome for biomedical applications

(In collaboration with CSIR-IICT, Hyderabad; Duration: January 2025 - December 2027)

Pawan Shukla, Ramesha A, Sanjit Kanjilal*, Rajkumar Banerjee*, T Ravinder*

*CSIR-IICT, Hyderabad

Objectives

1. Extraction and characterization of silkworm pupal oil
2. Isolation and characterization phospholipids from silkworm pupae for use in liposomal drug delivery systems.
3. Physicochemical/ biophysical characterization of silkworm phospholipid in various liposomal systems (both cationic and neutral liposomes) and their subsequent biological evaluation

A liposome is a small spherical vesicle made up of one or more lipid bilayers, frequently employed as a delivery system in medicine and cosmetics. Their unique ability to encapsulate and transport drugs or active ingredients to targeted areas within the body makes them highly valuable. Liposomes can be engineered to be biocompatible, biodegradable, and non-toxic, with sizes ranging from tens to hundreds of nanometers in diameter, making them particularly suitable for targeted drug delivery in pharmaceuticals.

Phospholipids are commonly used in liposome formulation. Silkworm pupal oil derived from the pupae of silkworm during silk production. Dried desilked pupae is a good source of oil (Triglycerides). An interesting aspect of pupal oil is the presence of 2.5-3.0% phospholipids. The innovative approach of the proposed project is the utilization of natural silkworm pupal phospholipids for the preparation of liposomes. Recently, there has been growing interest in incorporating polyunsaturated fatty acids into liposome preparations due to their health benefits.

This project aims to explore the development of pupal phospholipid based liposomal formulation for biomedical applications.

8. SITo8o18SNC: Construction and Efficacy Assessment of a BmNPV Derived Bacmid System for Recombinant Protein Production in *Bombyx mori* Silkworms

(In collaboration with IBAB, Bengaluru; Duration: Jan 2025 - Dec 2027)

Rajal Debnath, G. Subrahmanyam, Himanshu Dubey, Sanjay Ghosh*

*IBAB, Bengaluru

Objectives

1. To construct a BmNPV-derived bacmid system designed for recombinant protein production in the *Bombyx mori* silkworm host.
2. To evaluate the efficacy of the developed BmNPV bacmid system by expressing a GFP reporter gene in the *Bombyx mori* host.

The Baculovirus Expression Vector System (BEVS) has become a crucial platform for vaccine production, gene therapy, and biotechnology. BEVS offers rapid scalability, post-translational modifications, and high safety, making it a preferred choice for biopharmaceutical production. Beyond cell lines, whole silkworms - larvae and pupae have also served as an efficient production platform using BmNPV and cysteine protease-depleted BmNPV mutants (**Fig. 11**). Silkworm based systems have successfully produced various recombinant proteins. Several studies highlighted the silkworms' potential as bioreactors for diverse biopharmaceutical proteins. The varied expression levels emphasize their advantages in yield and post-translational modifications, making them valuable alternatives for large-scale protein production. Silkworm pupae offer unique advantages such as prolonged survival at 4°C without the need for mulberry leaves or artificial diets. Differing capabilities of silkworm larvae and pupae in protein expression and modification, make them valuable tools for tailored biotechnological applications. The technology for using BmNPV based bacmid systems specifically tailored for silkworms is currently not commercially accessible to researchers in India. Further, different species of wild silkworms have varying phenotype which offers opportunity to explore the efficacy of recombinant protein production. The recently initiated project is aimed at establishing novel bacmid system for silkworms to harness the full biotechnological potential of silkworms as bioreactors.

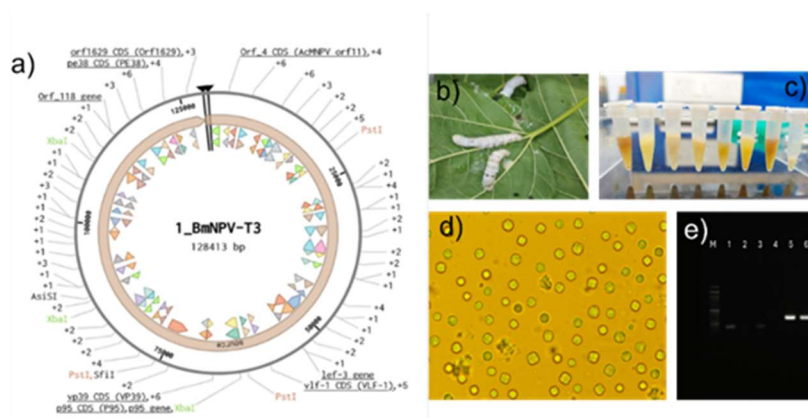


Figure 11: BmNPV virus infection, purification and DNA preparation. a) BmNPV circular genome (128 kb), b) infection of *B. mori* larvae c) hemolymph preparation from infected larvae d) purified NPV virus particles, e) PCR and gel confirmation of with polH specific marker

9. SITo8o16SNC: Cloning and Expression of Multifunctional Recombinant Fibroin Silk proteins for non-Textile applications

(In collaboration with Promolecular Sciences Pvt Ltd, Bengaluru;

Duration: January 2025 - December 2027)

Tulsi K. Naik, Rajal Debnath, Hemalatha*

*Promolecular Sciences Pvt Ltd, Bengaluru

Objectives

1. Cloning and expression of recombinant Fibroin silk protein into a suitable host system
2. Optimization of parameters for large scale production and purification of recombinant fibroin proteins
3. Validation of the activity of the Multi-functional Recombinant Fibroin Silk proteins for non-Textile applications

The silkworm *Bombyx mori* possesses a specialized silk gland responsible for producing, storing, and processing silk used for cocoon formation. During the late 5th instar, the gland secretes a protein-rich fluid that solidifies upon extrusion through the spinnerets to form silk fibers. Silkworm silk is composed primarily of fibroin consisting of heavy-chain (Fib-H) and light-chain (Fib-L) proteins linked via disulfide bonds and stabilized by the P25 protein and sericins (Ser1, Ser2, Ser3), which function as adhesive coating proteins.

Silk fibers from silkworms and spiders are valued for their exceptional tensile strength, biocompatibility, and biodegradability, making them highly attractive for biomedical and materials science applications. However, traditional silkworm silk contains sericin that can elicit immune responses and requires their removal during processing. Although regenerated silk fibroin is inexpensive and widely used, it often requires additional modification to achieve properties suitable for advanced biomaterial applications.

To overcome these limitations and expand the functional landscape of silk-based materials, genetic engineering is considered a promising strategy. Recombinant silk proteins incorporating silk-like motifs and functional peptide sequences enable the creation of dynamic, environmentally responsive, and mechanically robust biomaterials. This project is initiated with the aim of developing multi-functional silk proteins targeted for non-textile application.

OUTCOME OF CONCLUDED RESEARCH PROJECTS



1. ARPo8007MI: Biological and molecular characterization of virosis in Muga silkworm (*Antheraea assamensis* Helfer)

(In collaboration with MESSO; Duration: March 2022 - February 2025)

G. Subrahmanyam, K.M. Ponnuvel, K. Indira Kumar*, Abhishek Singh[#]

*P4 unit, Muga Eri Silkworm Seed Organization (MESSO), Tura, Meghalaya.

[#]P3 Unit, Muga Eri Silkworm Seed Organization (MESSO), Nongpoh, Meghalaya.

Objectives:

1. To characterize the viral pathogen of *Antheraea assamensis* Helfer
2. To study the epidemiology of the virosis in *Antheraea assamensis* Helfer
3. To study the resistance inheritance pattern for virosis in *Antheraea assamensis* Helfer

Cypoviruses (CPVs) infect more than 250 insect species across the orders Lepidoptera, Hymenoptera, Diptera, and Coleoptera. These viruses primarily target the midgut epithelial cells of insects. CPVs are double-stranded RNA viruses with segmented genomes comprising 10–12 segments, distinguished by their RNA profile and host specificity. CPV infections have been reported in *Antheraea* species, including *A. mylitta*, *A. proylei*, and *A. assamensis*. Despite recurrent viral outbreaks in Muga silkworm populations, comprehensive biological and molecular characterization of the causative virus remains lacking. This project aims to identify the viral pathogen, elucidate its infection mechanism, and determine its phylogenetic relationship with other known Cypoviruses. These insights are crucial for developing effective diagnostic tools, implementing timely disease management strategies, and guiding selective breeding programs for virus-resistant silkworm strains.

Results

The hemolymph and gut juice from infected Muga cadavers were observed under light microscope to confirm polyhedral structures. Scanning electron microscopy revealed distinct occlusion bodies (OBs) with polyhedral symmetry and variable dimensions ranging from 2 to 5 μm , while transmission electron microscopy confirmed the presence of icosahedral virions of approximately 60 nm embedded within a crystalline polyhedral matrix (**Fig. 12**). Electrophoretic analysis shows the typical ten-segmented RNA profile of Cypoviruses, with segments ranging from 3.8 to 1.1 kb, corresponding to essential structural and non-structural genes (**Fig. 13**). Phylogenetic analysis depicted that AaCPV4 was the closest to AmCPV4.

Bioassay studies demonstrated a clear dose-dependent pathogenic response in infected larvae, with 100% mortality achieved by day 12 post-inoculation at a concentration of 2.4×10^8 OBs/mL. The median lethal concentration (LC_{50}) was determined to be 1.96×10^5 OBs/mL, supported by a strong model correlation ($R^2 > 0.95$), confirming high virulence.

Tissue-specific analysis of viral RNA revealed the midgut as the primary site of replication, showing the highest RdRp gene copy number ($\log 9.65 \mu\text{g}^{-1}$ RNA), followed by the hindgut, hemolymph, and Malpighian tubules, whereas the silk gland exhibited minimal viral replication ($\log 4.45 \mu\text{g}^{-1}$ RNA). Virome sequencing established the complete genome size of approximately 24.9 kb, encompassing ten distinct dsRNA segments, each encoding a single open reading frame. Structural and functional annotation revealed high sequence similarity to Cypovirus 4 isolates, and all sequences were deposited in GenBank (accession numbers OR829953.2–OR829962.2).

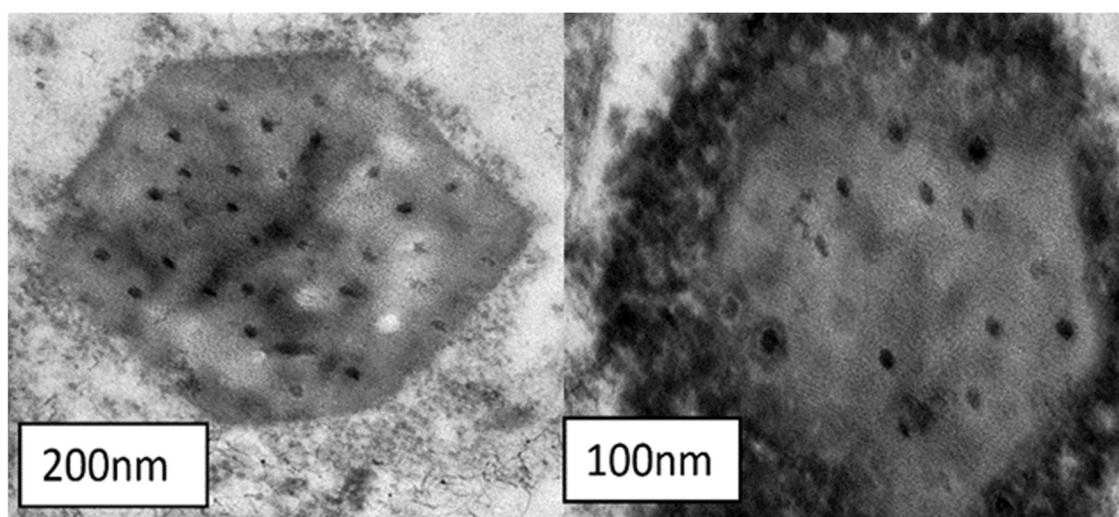


Figure 12: Transmission electron micrographs (TEM) of AaCPV4 at different magnifications, showing individual polyhedral structures with icosahedral virions embedded within the polyhedral matrix.

Cross-infectivity assays confirmed host specificity, as no infection was observed in other lepidopteran species, verified through PCR and qRT-PCR analyses. Comparative analysis for the presence of an additional segment (S-11) reported in *A. mylitta* CPV-4 showed its absence in both *A. assamensis* and *A. mylitta* isolates, confirming a ten-segmented genome organization. Expression of the AaCPV-4 polyhedrin gene in *E. coli* BL21 Codon Plus cells resulted in a recombinant 42.28 kDa protein (including vector tags), corresponding to a native 27.18 kDa polyhedrin of 237 amino acids, validated through sequencing (GenBank accession OQ789560.1).

Transcriptome profiling of infected tissues revealed significant differential gene expression, with 185 genes in the fat body (63 upregulated and 122 downregulated) and 283 in the midgut (100 upregulated and 183 downregulated). The differentially expressed genes were categorized based on molecular, biological, and cellular functions, highlighting the activation of antiviral immune pathways and providing valuable insight into the host–virus interaction dynamics in *A. assamensis*. We could not find resistance inheritance pattern for virosis in *A. assamensis* Helper

Outcome and utility of the project:

This study represents the first comprehensive report on the identification of the viral pathogen infecting the Muga silkworm (*Antheraea assamensis*), identified as Cypovirus antheraea (AaCPV-4). The complete genome sequence of AaCPV-4, comprising ten double-stranded RNA segments, is deposited in the NCBI GenBank under accession numbers OR829953 to OR829962, and the high-throughput RNA-Seq raw reads are available in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1139378. The PCR and qRT-PCR-based diagnostic assays developed in this study provide reliable and sensitive tools for the rapid detection of AaCPV-4 infection in Muga silkworms.

Considering the virus's remarkable host specificity infecting only *Antheraea* species such as *A. assamensis*, *A. mylitta* and *A. proylei*, further investigation into host-pathogen interactions could facilitate the development of disease-tolerant or resistant silkworm breeds. Additionally, the disinfectant formulation standardized in this work demonstrated strong virucidal efficacy by completely dissolving the viral occlusion bodies and degrading the viral RNA, thereby serving as a promising measure for disease control in rearing facilities. Furthermore, the multiplex PCR assay was successfully validated using field samples of *A. assamensis*, establishing it as a valuable and efficient diagnostic and surveillance tool for simultaneous detection of both pebrine and virosis diseases in Muga sericulture.

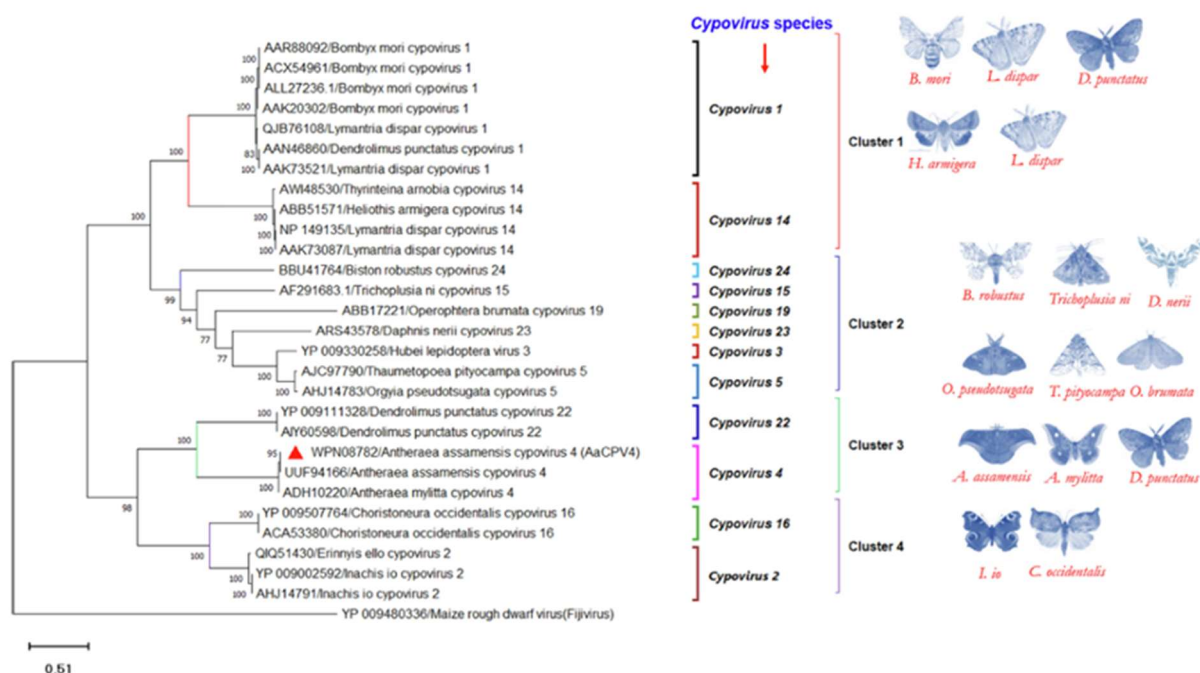


Figure 13: Maximum likelihood phylogenetic tree of AaCPV4 constructed using deduced amino acid sequences of the RNA-dependent RNA polymerase encoded by viral genomic segment 2.

2. AIBo8007MI: Development and evaluation of eri silkworm (*Samia ricini* Donovan) breeds/hybrids with improved productivity

(Collaboration with CMER&TI, SSTL, P2-BSF-Topatoli & ESSO-Hosur; Duration: March 2022-February 2025)

R. Debnath, Ramesha A, Himanshu Dubey, Prateesh Kumar PM², Lalitha Natarajan³ & Malashree N⁴

¹CSB, Bangalore, ²ESSO-Hosur, ³P2-BSF-Topatoli, ⁴SSTL

Objectives:

1. Generation of stable breeding lines of Eri silkworm strains/morphotypes
2. Utilization of molecular markers to develop and characterize eri hybrids with economic value

Eri silkworm populations consist of four morphotypes based on body color and larval markings: yellow plain (YP), greenish blue plain (GBP), yellow zebra (YZ), and greenish blue zebra (GBZ). These morphotypes coexist within natural populations, and it is hypothesized that they differ in silk yield and quality. To overcome the limitations posed by mixed populations, sib-mated, non-segregating (homozygous) lines were developed for each morphotype, enabling isolation of phenotype-specific genetic effects and accurate assessment of silk production traits.

Analysis of the stabilized lines revealed a significant association between larval markings and increased cocoon and pupal weight. Marked morphotypes (YZ and GBZ) showed significantly higher mean cocoon weights than plain morphotypes (YP and GP) ($p < 0.05$), suggesting that genetic loci governing larval markings may be linked to enhanced silk production (**Fig. 14**).

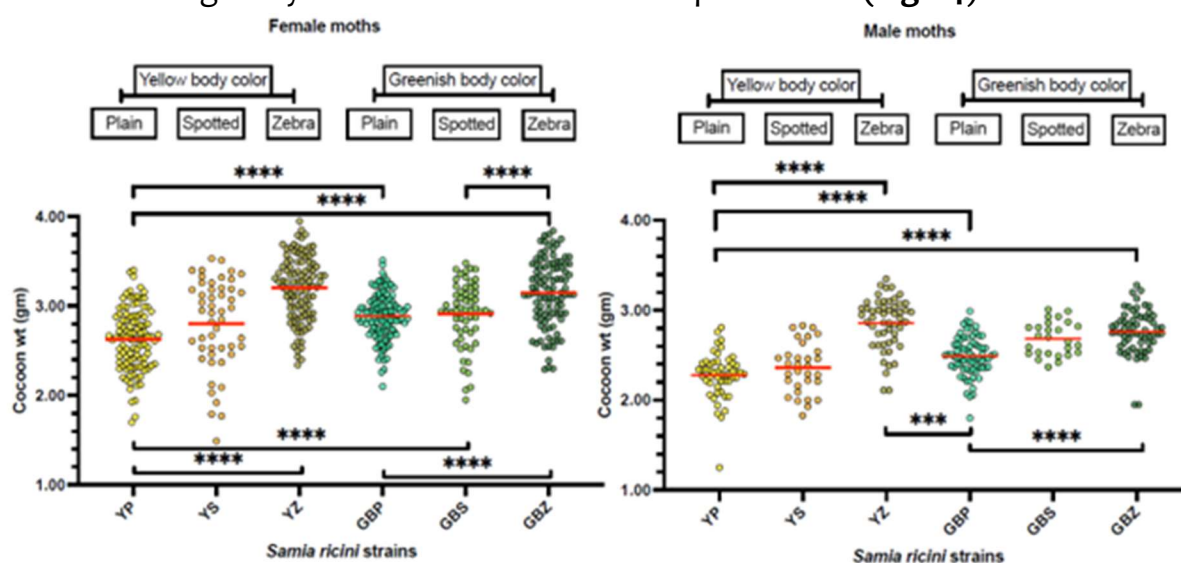


Figure 14: Distribution of cocoon wt amongst the silkworm populations at G₇ generation indicates YZ and GBZ lines have significantly higher cocoon wt ($p < 0.05$) compared to plain morphotypic lines.

Genetic crossing experiments indicated that greenish body color is dominant over yellow, although segregation was observed only for body color and not for the marking phenotype (**Fig. 15**). To assess the genetic impact of sib-mating, ddRAD-seq genotyping was performed across all stabilized lines and the C2 breed. After filtering, 82,498 high-quality SNPs were retained for population genetic analyses. Genome-wide SNP profiling and PCA revealed clear genetic clustering of each line, confirming increased homozygosity due to sib-mating. STRUCTURE analysis further showed >95% ancestry purity in each morphotypic line by generation 12, demonstrating successful genetic stabilization (**Fig. 16**).

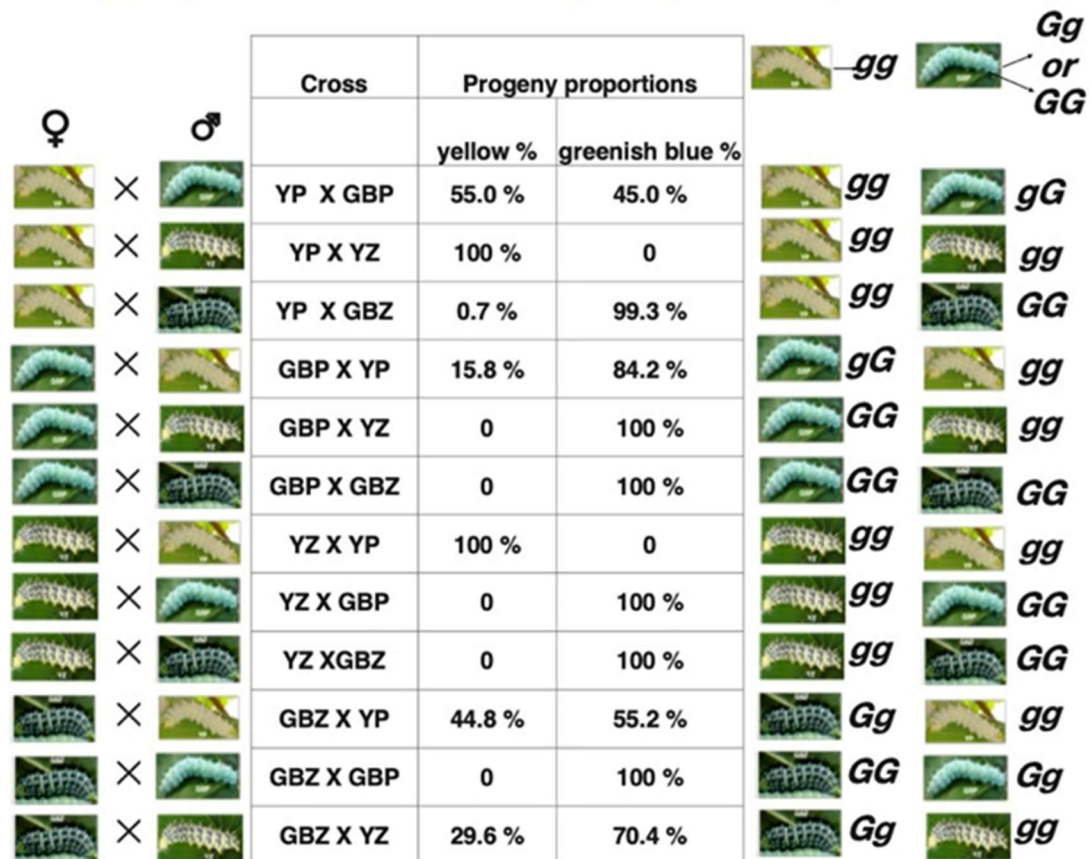


Figure 15: Crosses of stabilized morphotypes and progeny proportions indicate greenish body color trait is dominant over yellow body color. Table shows outcome of progeny proportions (%) scored from cellularly reared DFLs for each cross combination.

Outcome and utility of the project:

We developed genetically distinct morphotypic lines of *Samia ricini* (eri silkworm) through a systematic approach of recurrent phenotypic selection followed by multiple generations of sib-mating. Four stable morphotypes were derived based on larval body color and larval marking patterns: YP (yellow plain), GP (greenish plain), YZ (yellow zebra), and GZ (greenish zebra). These stabilized, non-segregating lines were evaluated for cocoon and pupal weight, and the results showed that the marked morphotypes (YZ and GZ) consistently exhibited significantly higher cocoon and pupal weights compared to their unmarked counterparts (YP and GP). These differences remained largely consistent across rearing cycles, suggesting that the marking phenotype may be genetically linked with loci influencing yield-associated traits.

To characterize the underlying genetic structure, we performed ddRAD based sequencing on representative individuals from each line. The data revealed the presence of genetically isolated haplotypes that may be useful in capturing body color-associated regions (YP vs GBP) and pattern associated regions (GBZ vs YZ). These genetic resources will be further used in eri breeding work to characterize the yield specific traits that are associated with significant marker loci.

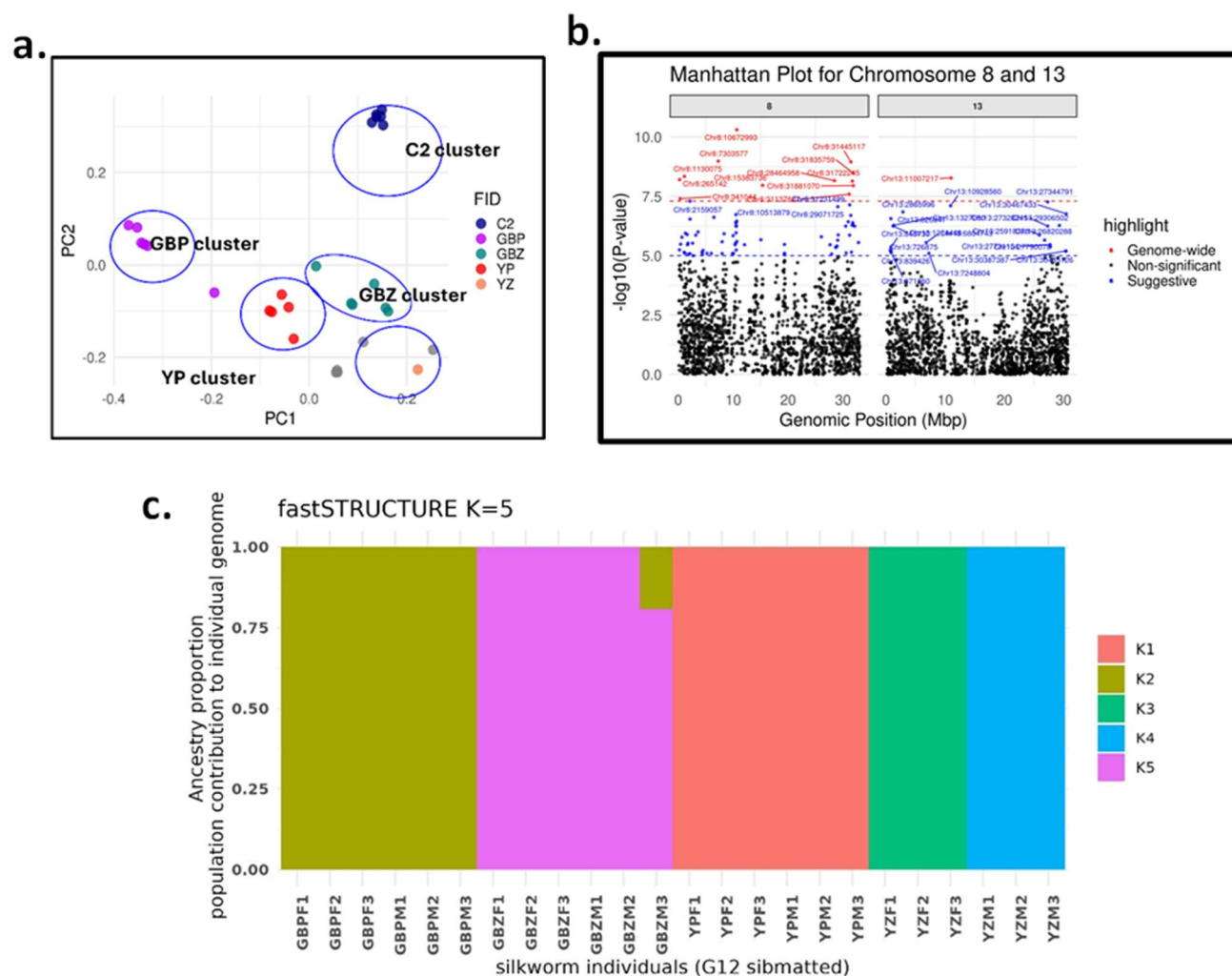


Figure 16: a) PCA plot of individual cluster from each of the breeding lines. b) Manhattan plot showing SNP loci on chromosome 8 & 13. c) faststructure plot indicates genetic purity among the individual from each sib-matted population based on genome wide SNP based profile.

3. AICo8009CN: Profiling of lipid, protein and carbohydrate of mulberry mealybug *Maconellicoccus hirsutus* (Green) for its control

(Collaboration with CSR&TI Mysore; Duration: April 2022-March 2025)

Pawan Shukla, Ramesha A, Himanshu Dubey, Mahiba Helen*, Kesavan Subaharan[#]

*CSR&TI, Mysore; [#]NBAIR, Bengaluru

Objective:

1. Analysis of carbohydrate, protein and lipid profile of Mulberry mealybug.

Mulberry is an economically important tree for the sericulture industry, providing livelihoods to many small and marginal farmers. However, maintaining pest control without compromising yield remains a major challenge. Among sap-sucking insects, the pink mealybug *Maconellicoccus hirsutus* is a serious pest of mulberry and several agricultural, horticultural, and ornamental crops in tropical and subtropical regions. It feeds on phloem sap using piercing-sucking mouthparts and injects watery saliva into tender tissues, causing leaf malformation, stunted shoot growth, shortened internodes, and branch dieback. In mulberry, these symptoms are known as *tukra* disease, which significantly reduces both the quality and quantity of foliage, thereby affecting silkworm rearing. Mealybugs protect themselves from pesticides and natural enemies by secreting a thick waxy coating. Continuous phloem feeding results in the excretion of carbohydrate-rich honeydew due to low nitrogen availability in plant sap. To regulate osmotic balance, mealybugs possess a specialized filter chamber that facilitates sugar excretion. Excess honeydew promotes the growth of black sooty mold on leaves and stems, further impairing plant health.

Chemical control is largely ineffective because insecticides cannot penetrate the waxy covering and egg ovisacs. Although biological control using predators and parasitoids is effective, its success is often compromised by ants that form mutualistic associations with mealybugs by harvesting honeydew and protecting them from natural enemies. This necessitates alternative management strategies. The present study aims to characterize the lipid, protein, and carbohydrate composition of the mulberry mealybug, including its wax layer, saliva, and honeydew.

Honeydew analysis:

Freshly excreted honeydew of female pink mealybugs, initially transparent and watery, was collected prior to oxidation. Samples were hydrolyzed, derivatized to trimethylsilyl derivatives, and analyzed by GC-MS using a DB-5MS column with helium as the carrier gas. GC-MS analysis revealed a complex mixture comprising sugars, sugar alcohols, organic compounds, and organic acids, including arabinose, D-glucopyranose, D-mannose, glycerol, myo-inositol, lactic acid, levoglucosan, levulinic acid, gluconolactone, mannonic acid lactone, and pentanoic acid. Among these, four were monosaccharides, five organic compounds, and three sugar alcohols (**Table 1**).

Table 1: List of compounds identified in the honeydew of pink mealybug

Sl. No.	Compound Name	Type of Compound	Carbon atom	Functional group	Molecular Weight
01	Arabinose	monosaccharide	5C	aldehyde	150.13 g/mol
02	D-Glucopyranose/ Glucose	monosaccharide	6C	aldehyde	180.156 g/mol
03	D-Mannose	monosaccharide	6C	aldehyde	180.156 g/mol
04	levoglucosan	Organic compound	6C	hydroxy	162.14 g/mol
05	Gluconolactone	oxidized derivative of glucose	6C	hydroxy	178.14 g/mol
06	Lactic acid	Organic acid	3C	Carboxy and hydroxy	90.08 g/mol
07	Pentanoic acid	Organic compound	5C	Carboxy	116.11 g/mol
08	Levulinic acid	Organic compound	5C	Carboxy	116.11 g/mol
09	Mannonic acid, Lactone	Acidic compound	6C	hydroxy	178.14 g/mol
10	D Chiro-Inositol	Sugar alcohol	6C	hydroxy	180.16 g/mol
11	Myo-inositol	Sugar alcohol	6C	hydroxy	180.16 g/mol
12	Glycerol	Sugar alcohol	3C	hydroxy	92.09 g/mol

a. Wax analysis:

Wax from the pink mealybug (*Maconellicoccus hirsutus*) was collected under a stereo zoom microscope and analyzed as fatty acid methyl esters (FAMES) using GC-MS, with comparison to a standard mixture. The chromatogram showed prominent peaks corresponding to saturated and unsaturated long-chain fatty acids. GC-MS analysis identified eight major fatty acid components, with experimental retention indices closely matching reported values, confirming compound identities. The wax was dominated by saturated fatty acids, primarily stearic acid (C18; 41.95%), dodecanoic acid (C12; 24.70%), and tetradecanoic acid (C14; 12.52%). Unsaturated fatty acids included oleic acid (C18:1, ω -9; 8.88%) and linoleic acid (C18:2, ω -9, ω -12; 4.24%). Minor components comprised decanoic acid (1.18%), hexadecanoic acid (2.73%), and eicosanoic acid (1.49%).

b. Saliva Proteome:

Saliva from mealybug crawlers was collected using a non-destructive artificial diet system, followed by dialysis, protein precipitation, and SDS-PAGE analysis. Proteins were trypsin-digested, desalted, and analyzed by LC-MS/MS. Approximately 2,100 proteins were identified, of which 55 were confidently assigned as insect-derived and significantly expressed. These proteins localized to diverse cellular compartments and were involved in essential biological processes, including development, digestion, detoxification, immunity, chitin metabolism, hormone regulation, energy metabolism, and cytoskeletal maintenance. Expression profiling of selected genes further validated these findings (**Fig. 17**). Notably, odorant-binding protein, cathepsin B endopeptidase-like protein, legumain, and heat shock protein 70 emerged as potential RNAi targets for pink mealybug management.

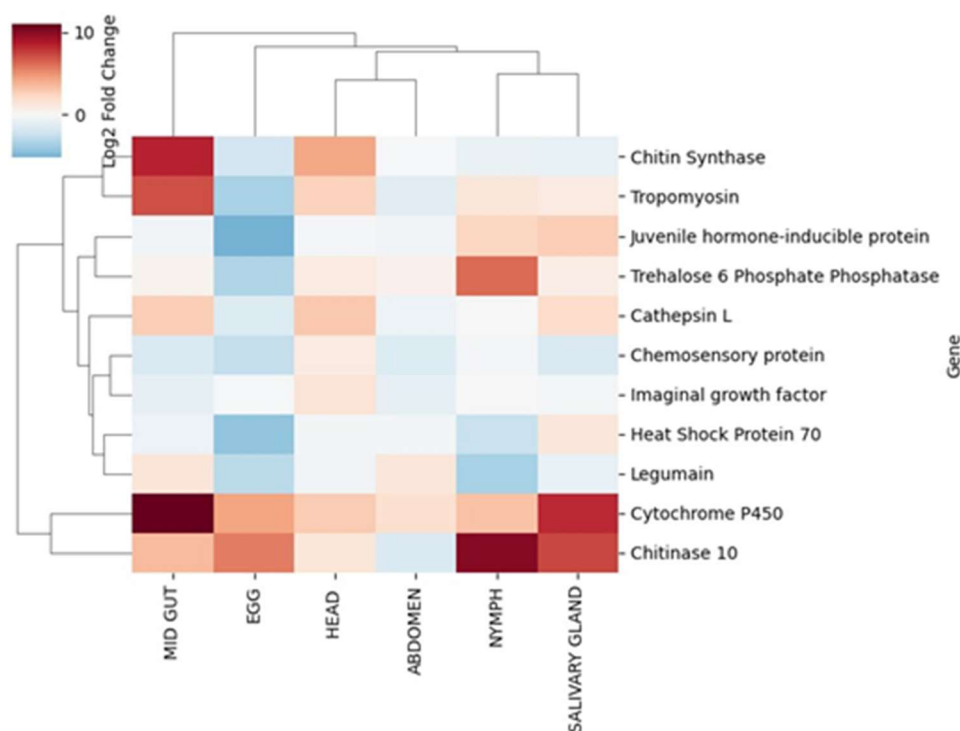


Figure 17: Gene expression analysis of some selected genes observed during pink saliva proteome

c. Wax dissolving Bacteria:

To develop an alternative strategy for mealybug control, wax-degrading bacteria were isolated from soil. Serially diluted soil samples were plated on minimal agar medium supplemented with mealybug wax. Three bacterial isolates were selected, purified on Luria-Bertani agar, and preserved as glycerol stocks. The isolates were characterized using IMViC tests, and their wax-degrading ability was confirmed by the rhodamine assay.

Outcome and utility of the project:

This project provided comprehensive biochemical insights into the pink mealybug by characterizing its honeydew, wax, and saliva. Honeydew analysis revealed a complex mixture of sugars, sugar alcohols, organic compounds, and organic acids. Wax compositional profiling showed that saturated fatty acids-particularly stearic and dodecanoic acids-were dominant, with notable proportions of unsaturated fatty acids. Wax-degrading bacteria were also identified, offering potential for biological control. LC-MS/MS analysis of saliva uncovered 55 insect-derived proteins linked to essential physiological functions, with several-including odorant-binding protein, cathepsin B-like protein, legumain, and heat shock protein 70-emerging as promising RNAi targets. Collectively, these findings contribute valuable resources for developing targeted and eco-friendly management strategies for pink mealybugs.

TRANSFER OF TECHNOLOGY



Component 1: OFT for the evaluation of BmBDV resistant mulberry silkworm Double Hybrids at farmer's field.

(In Collaboration with CSRTI, Mysore; Duration July 2023 - June 2025)

Dr Tulsi Naik K S, Dr Ramesha A, Dr Ranjini M S*

*CSRTI, Mysore

Objective:

1. To evaluate the FC1R x FC2R double hybrids resistant to BmBDV (Viral Flacherie) at farmers locations in southern region of India.

Viral diseases of silkworm cause a major problem to sericulture as they account for almost 70 per cent of total crop loss. The two major viral diseases include grasserie caused by *Bombyx mori* Nuclear polyhedrosis virus (BmNPV) causes 42.60 per cent crop loss and viral flacherie caused by *Bombyx mori* Infectious flacherie virus (BmIFV) causes 47.90 per cent crop loss, *Bombyx mori* Densonucleosis virus type 1 (BmDNV-1) and *Bombyx mori* Densonucleosis virus type 2 (BmDNV-2) causes 9.50 per cent crop loss. The survey of Flacherie disease on silk worm was carried out, during the year 2010-11 through five districts (Akola, Amravati, Buldhana, Washim and Yavatmal) of Western Vidarbha regions in Maharashtra. As many as 250 silkworm rearing centers located in various villages were surveyed. During study year the incidences of Flacherie was (3-10%) in spring and monsoon season.

Technology Developed and the Information generated

The overall results indicate that significant improvement in the survivability of the BmBDV resistant double hybrids was observed in all the trail locations, particularly during Rainy and Autumn seasons while in spring season the survivability was similar in both control and BmBDV resistant hybrids **Fig. 18**. The Introgressed BmBDV (bidensovirus) resistance in commercial double hybrid (FC1R x FC2R) of bivoltine silkworm through marker assisted breeding resulted in average yield of 74 kgs/100 dfl compared to 70kgs/100 dfl in control (FC1 x FC2) during autumn season in Sothern regions (Ananthapur, Mulugu, Salem, Chitradurga) and RSRS Ananthapur which showed a significant average >10% increase in yield/100DFLs irrespective of seasons. Further, the decrease in survival in the control (July-october) may be attributed to severe rain during the rearing resulted in increase in humidity & variations in the temperatures throughout the day which are pre disposing factors for pathogen multiplication that had significant impact on the survivability in the control batches due to viral Flacherie and also multiple infections. The increase in survival in BmBDV resistant double hybrids may be attributed to the presence of *nsd-2* resistance gene responsible for resistance against the BmBDV infection in the host. Therefore, we could observe variations in the yield/100DFLs. The Effective rearing rate (ERR %) of the BmBDV resistant batches were significantly high during all the seasons. However, in some stations it was observed that though the ERR% was higher in the BmBDV resistant double hybrids, the yield /100DFLs was comparatively less, this may be attributed to the fact that the yield/100DFLs is calculated based on the cocoon weight.

Therefore, though the survival/ERR was higher in the BmBDV resistant double hybrids, the cocoon weight was comparatively less and therefore resulted in decreased yield Fig. 19. However, the post cocoon parameters are at par with the control double hybrids indicating no compromise in the cocoon quality parameters. The on-station trials highlight on the importance of survivability in the test double hybrid which was developed for BmBDV resistance through marker assisted selection under natural rearing conditions in the field. The results evaluated at four different RSRs substantiate the validation for improved survivability of the test double hybrid when compared to control during Rainy and Autumn seasons with a range of 3-8% without any significant compromise in the yield parameters except for the cocoon weight between control and test hybrid (Table 2).

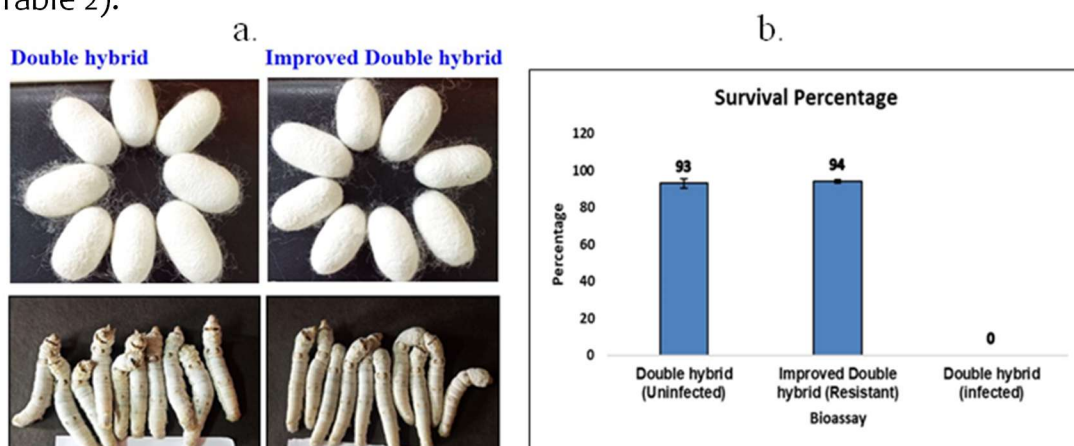


Figure 18: a. The larvae and the cocoons of the improved double hybrids taken for the on-farm trials. b. The survival percentage of control and the BmBDV resistant double hybrids

Table 2: Performance of the BmBDV resistant double hybrid during the on-farm trials

Sl. No.	Race	Egg/ Dfl	Survival %	Yield/10000 Larvae		Yield/ 100 DFLs (Kg)	Single cocoon wt. (g)	Single shell wt. (g)	Shell Ratio %
				By No.	By Wt. (Kg)				
RSRS Ananthapur	FC1R X FC2R	518	85.56	8560 (85.60%)	15.56	77.65	1.818	0.41	22.65
	FC1 X FC2 (Control)	485	73.61	6945 (69.45%)	12.10	60.40	1.744	0.37	21.67
RSRS Salem	FC1R X FC2R	566	96.75	9650 (96.50%)	16.19	84.58	1.624	0.34	21.07
	FC1 X FC2 (Control)	523	93.70	9377 (93.77%)	17.08	88.35	1.871	0.40	21.72
RSRS Mulugu	FC1R X FC2R	526	90.46	8961 (89.61%)	15.18	75.90	1.750	0.36	20.57
	FC1 X FC2 (Control)	479	86.46	8488 (84.88%)	14.20	71.00	1.810	0.35	19.33
REC Chitradurga	FC1R X FC2R	551	81.31	8133 (81.33%)	13.53	72.02	1.664	0.35	21.11
	FC1 X FC2 (Control)	558	81.59	8131 (81.31%)	14.41	67.67	1.771	0.38	21.49

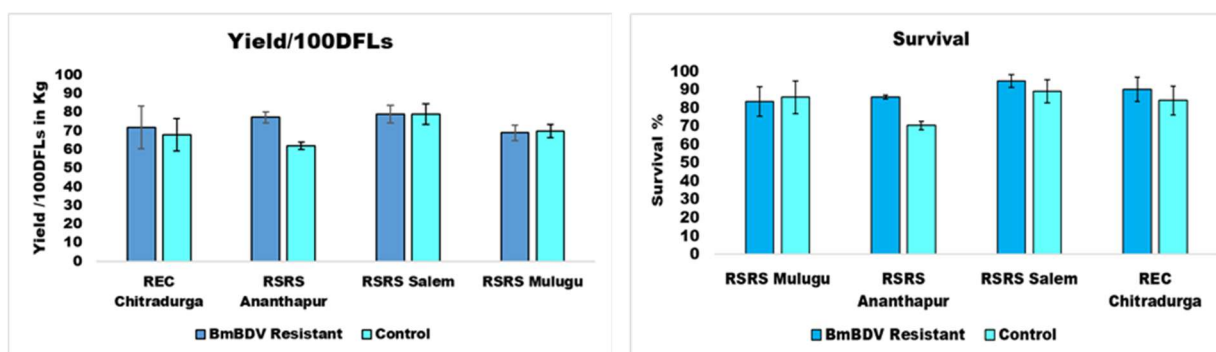


Figure 19: The survivability and the yield/100DFLs of the BmBDV resistant double hybrids at farmers field under the RSRs/REC stations under CSRTI, Mysore

Outcome of the trials

The rationale of the study highlights on the importance of survivability in the improved double hybrid which was developed for BmBDV resistance through marker assisted selection. The overall results indicate improved survivability of the BmBDV resistant double hybrids during the on-farm trials particularly during rainy and autumn seasons. Further, the yield /100DFLs in BmBDV resistant double hybrids was higher compared to control without any compromise in the quality parameters. The improved double hybrid with the disease resistant trait has the potential to enhance the farmers profit by 3-5% particularly during the rainy and autumn seasons when the disease incidences are prominent (**Table 3**).

Table 3: Progress achieved against the set target

Name of the Technology	No of DFLs	Unit Cost (Rs.)	No. of locations	No. of stakeholders	Outcome
OFT for the evaluation of BmBDV resistant mulberry silkworm Double Hybrids at farmer's field. Hybrid to be tested under the OFTs: FC1R X FC2R	Target 20000 DFLs Achieved 17700 DFLs	11.8	10	50	The improved hybrids performance during filed trials showed an average yield of 74 kgs/100 dfl in BmBDV resistant hybrids compared to 70 kgs/100 dfl in control rearing during autumn season. Overall performance of the BmBDV resistant hybrids showed avg 3-5% increase in yield/100DFLs, However, BmBDV resistant hybrids showed 5-10% increased survivability

Component II: OST for evaluation of BmBDV resistant mulberry silkworm hybrids at CSB/Dos stations.

Duration (July 2023-June 2025)

Investigators: Tulsi Naik K S, Ramesha A, ISBR Bangalore
Rahul K, Mihir Rabha, CSRTI, Berhampore, B. Vijaya Naidu, REC Bagmara,
Mothabari, Harish Babu, RSRS Kalimpong

Objective:

To assess the field performance of SK6RxSK7R resistant to BmBDV (Viral Flacherie) at designated stations under CSRTI, Berhampore

The silkworm *Bombyx mori* L (Lepidoptera: Bombycidae) is an economically important insect species which produces silk cocoons that have been bred in captivity for around 5000 years and now have been domesticated completely. Bivoltine silkworms garner great attention for their superior quality as well as high quantity of silk, but are susceptible to disease causing biotic as well as abiotic factors which affect the cocoon crop yield. High temperature and humidity are the major important factors which decrease the crop production in tropical zones like West Bengal and Jharkhand (North eastern) states. However, efforts were made for the development of several robust and productive bivoltine hybrids and time to time considerable achievements have also been made for developing thermo tolerant bivoltine breeds suitable for tropical climatic conditions. The two important thermo tolerant breeds i.e., SK6 and SK7 have been utilized as bivoltine parents in the development of cross breeds (Mi x Bi). To fulfil the demand of bivoltine seed cocoons for preparation of Multi x Bi hybrids in the North-eastern regions like West Bengal and neighbouring states, the CSRTI, Berhampore developed SK6 x SK7 as bivoltine foundation cross which could be reared successfully in all seasons. The SK6 x SK7 foundation cross is used as parent to develop Ni x (SK6 x SK7), which is a commercial breed recently introduced in the field and produces a yield up to 50kg/100 dfls.

The on-station trials conducted at three designated locations showed a significant increase in survival and the yield /100DFLs in the SK6R x SK7R single hybrids compared to control. The singly hybrids were tested during three different seasons as per the crop schedule i.e., Agrahyani, Falguni & Baisaki seasons. The performance of the hybrids during all the seasons were significant with the highest survival of 11% percent compared to control was reported at the RSRS Kalimpong, while the remaining stations showed an average of 2-6% increase in survival against the control. The BmBDV resistant hybrids showed an average of 3-5% increase in Yield/100DFLS in all the stations irrespective of the season (**Fig. 20, Table 4**).

Table 4: On station performance of BmBDV resistant single hybrids at RSRS/REC stations

RSRS/REC Test Centres	Race	Egg/ Dfl	Survival %	Yield/10000 Larvae	Yield/100 DFLs	Single cocoon wt. (g)	Single shell wt. (g)	Shell Ratio %
				By No.				
RSRS Kalimpong	SK6R X SK7R	476	51.6	5110	39.5	1.47	0.243	16.53
	SK6 X SK7 (Control)	460	40.0	4854	36.6	1.51	0.247	16.32
REC Baghmara	SK6R X SK7R	498	66.6	6064	44.21	1.50	0.253	16.82
	SK6 X SK7 (Control)	510	62.3	5684	38.66	1.49	0.253	16.96
CSRTI Berhampore	SK6R X SK7R	543	69.6	6896	45.33	1.48	0.245	16.53
	SK6 X SK7 (Control)	553	67.33	6671	40.33	1.47	0.243	16.50

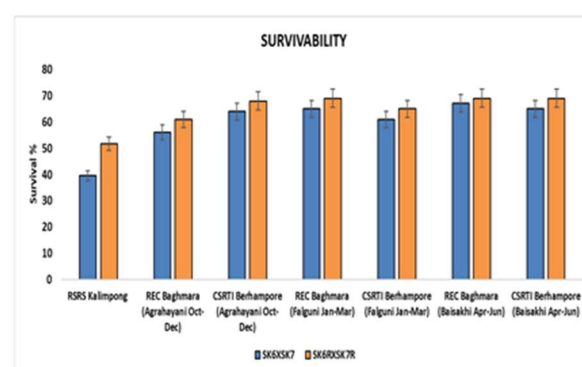
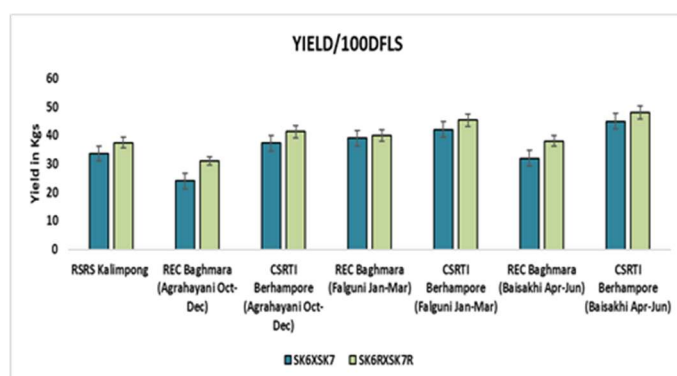
**Figure 20:** Performance of the BmBDV resistant single hybrids during different seasons at designated RSRS/REC stations under the CSRTI, Berhampore.

Table 5: Performance of BmBDV resistant single hybrids during the OST Trials

Sl. No	Name of the Technology	Unit Cost (Rs.)	RSRSs	Total In lakhs	Outcome
1	OST for evaluation of BmBDV resistant mulberry silkworm hybrids at CSB/Dos stations	5DFLs control SK6XSK7 and BmBDV SK6RX & SK7R resistant lines	03 Stations 1. RSRS Kalimpong 2. REC Baghmara 3. CSRTI, Berhampore	1.04 lakhs	BmBDV resistant SK6R x SK7R hybrids resulted in average yield of 43.05 kg/100 dfl compared to 35.5 kg/100 dfl in Control (SK6 x SK7) in West Bengal region.

Outcome of the on-station trials

The overall performance of the BmBDV resistant SK6R x SK7R hybrids resulted in average yield of 43.05 kg/100 dfl compared to 35.5 kg/100 dfl in Control (SK6 x SK7) in West Bengal region. The single hybrids can be further evaluated at farmers field to validate the performance of the hybrids under farmers rearing conditions (**Table 5**).

TRAINING, WORKSHOP & OTHER ACTIVITIES



TRAINING

The Institute organized a five-day training programme titled “Hands-on Training on Real-Time PCR” from 24th to 28th June 2024 under the Platinum Jubilee Year celebrations of the Central Silk Board (CSB). The programme was conducted at CSB-Institute for Seri-Biotechnological Research (CSB-ISBR), Kodathi, with the objective of strengthening both practical skills and conceptual understanding of advanced molecular techniques relevant to sericulture research. The training featured a well-structured blend of theoretical lectures and intensive hands-on practical sessions covering genomic DNA and RNA isolation, gel electrophoresis, primer design, PCR, real-time PCR (qRT-PCR), cDNA synthesis, data analysis, and basic bioinformatics, delivered by experienced CSB scientists. A total of 10 participants, including CSB scientists, research scholars, project students, and Ph.D. scholars from Jain University, actively participated in the programme. The training significantly enhanced the participants’ technical competence in molecular diagnostics and research applications, thereby contributing to capacity building and skill upgradation in biotechnology with specific relevance to sericulture research.



RAC Meeting

The 33rd meeting of the Research Advisory Committee (RAC) of the Institute was held on 15th July 2024 at the Seri-Biotech Research Laboratory, Kodathi, Bengaluru, under the Chairmanship of Prof. H. S. Subrahmanya, Former Director, IBAB, Bengaluru, and currently Professor & Dean, School of Biosciences and School of Mathematics and Natural Sciences, Chanakya University, Bengaluru. The Committee reviewed the follow-up actions on the recommendations of the previous RAC meeting, evaluated the outcomes and progress of ongoing research projects, and examined the concept notes of new research proposals for approval. The meeting also deliberated on trial of technologies, training programmes, and other institutional research activities. The valuable suggestions and guidance provided by the RAC members are expected to strengthen the quality, relevance, and impact of the Institute's research programmes and ensure their effective alignment with national priorities in sericulture and biotechnology research.



NABL Workshop

An awareness programme on NABL ISO/IEC 17025:2017 was organized by CSB-ISBR on 19 December 2024 at the Central Office, Central Silk Board (CSB), Bengaluru. The programme was inaugurated by Shri P. Sivakumar, IFS, Member Secretary, CSB, and Dr. S. Manthira Moorthy, Director (Technical), CSB. The programme was facilitated by Mr. Ranjith Kumar, NABL (Bengaluru Region), along with officers and officials from NABL, Bengaluru, who participated as resource persons. The main theme of the workshop was ISO/IEC 17025:2017 and its implementation in testing and calibration laboratories. The programme aimed to provide participants with a comprehensive understanding of ISO/IEC 17025:2017 requirements and NABL accreditation procedures, while strengthening laboratory competence in both technical and quality management systems. Emphasis was also placed on preparing laboratories for NABL assessments, promoting continual improvement, and ensuring sustained compliance with ISO/IEC 17025:2017 requirements.



राजभाषा कार्यान्वयन - OFFICIAL LANGUAGE IMPLEMENTATION

राजभाषा कार्यान्वयन के अंतर्गत किये गए कार्यों की प्रगति इस प्रकार है:

The progress achieved under Implementation of Official Language is as follows:

- ❖ संस्थान ने अनुच्छेद 3(3) के प्रावधानों का अनुपालन करते हुए सभी दस्तावेज द्विभाषी में जारी किए गए।

The Institute has followed the provisions of article 3(3) and issued all documents in bilingual.

- ❖ संबंधित क्षेत्रों में हिंदी में पत्राचार के लक्ष्य (जोन ए में 100%, जोन बी में 100% और जोन सी में 55%) प्राप्त किए गए थे। साथ ही फाइलों में हिंदी टिप्पणियों के संबंध में भी लक्ष्य हासिल किए हैं।

The targets for correspondence in Hindi in respective zones were achieved (100% in Zone A, 100% in Zone B and 55% in Zone C. It has also achieved the targets with respect to Hindi notings in the files.

- ❖ राजभाषा अनुभाग, केंद्रीय रेशम बोर्ड द्वारा आयोजित हिंदी कार्यशालाओं में भाग लिया।

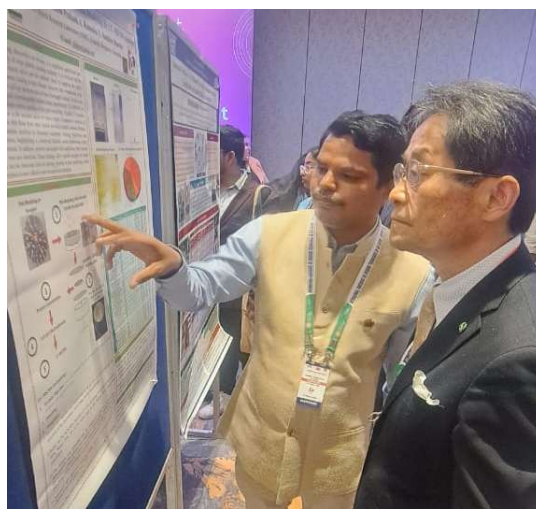
Attended Hindi workshops, organized by Official Language Section, Central Silk Board.

- ❖ हिंदी पखवाड़ा 02 से 17 सितंबर 2024 तक मनाया गया और विभिन्न कार्यक्रम आयोजित किए गए।

Hindi Pakhwada was celebrated from 2nd -17th September 2024 by conducting various events.

Meeting / Workshop / Conference / Symposium / Seminar Attended by the Scientists

1. Dr. Himanshu Dubey, Scientist-C attended the XXVII International Congress of Entomology, 25th to 30th August, 2024 at Kyoto, Japan
2. Dr. Pawan Shukla and Dr. Tulsi Naik attended the राष्ट्रीय राजभाषा तकनीकी सेमिनार on 24th August 2024 at CTRTI, Ranchi.
3. Dr. Tulsi Naik attended 15 days training program on “Processing Technology and Innovation Design of Modern Silk Products” at ZSTU, China.
4. Dr. Rajal Debnath attended Workshop on “Applications of remote sensing and GIS in sericulture” organized by North Eastern Space Applications Centre (NESAC), Department of Space, Govt of India, Umiam, Meghalaya and sponsored by Central Silk Board, Gov of India from 24th June 2024 to 05th July 2025
5. Dr. Pawan Shukla attended the International Conference on Emerging Technologies in Silk Sector -SILKTECH 2025 on 17th February 2025 at Bharat Mandapam, New Delhi
6. Dr. Subrahmanyam G., Scientist-D delivered the invited lecture at Kakatiya University, Telangana
7. Dr. Subrahmanyam G., Dr. Tulsi Naik and Dr. Himanshu Dubey attended 2nd International Conference on 'Emerging Concepts in Biotechnological Innovations' (2nd ICECBI-2025) from 26th to 28th of February, 2025 organized by SRM University.



AWARDS / ACCOLADES

1. **Dr. K. S. Tulsi Naik**, Scientist D received **Best CSB Scientist Award (R&D)** for the year 2023-2024. on the occasion of platinum jubilee celebration of Central Silk Board.
2. **Dr. G. Subrahmanyam**, Scientist D received the **Best innovation award** received at Second International Conference on Emerging Concepts in Biotechnological Innovations (ICECBI), SRM Institute of Science and Technology (SRMIST), Kattankulathur, Chengalpattu, Tamil Nadu, 26–28 February 2025
3. **Dr. Himanshu Dubey**, Scientist C received the “**Best Research Award**” at the 2nd International Conference on Emerging Concepts in Biotechnological Innovations (February 26–28, Chennai 2025), organized by SRM University, Chennai.
4. **Dr. Pawan Shukla**, Scientist D received **best Poster presentation award (Third Place)** in International Conference on Emerging Technologies in Silk Sector -SILKTECH 2025 on 17th February 2025 at Bharat Mandapam, New Delhi
5. **Dr. Pawan Shukla**, Scientist D received **Third Prize** in the All-India Hindi Self-Composed Poem Competition organized by the Central Silk Board (2024)
6. **Dr. K. S. Tulsi Naik**, Scientist D received **Consolation Prize** in the All-India Hindi essay Competition organized by the Central Silk Board (2024)



OTHER ACTIVITIES

Hindi Fortnight

The Hindi Fortnight was observed from 02nd-17th September 2024, featuring Hindi Day and a variety of competitions to celebrate the occasion. Additionally, Silk Day was commemorated on 20th September 2024, honoring the visionary Dr. Shyama Prasad Mookerjee for his significant contributions to the development of India's silk industry.

National Unity Day

The National Unity Day was observed on 31st October 2024 in which the staff of CSB-ISBR took pledge.

Vigilance Awareness Week

Vigilance awareness week was observed w.e.f. 30th Oct - 05th Nov 2024

World Environment Day

World environment day was observed on 06th June 2024. On this occasion, plantation drive was held.

Yoga Day

Yoga day was organized on 21st June 2024 in which all the staff of CSB-ISBR actively participated.





Sports activities on campus



Visit of school students to the institute



Signing of MoU



Scientists' visit abroad

COMMITTEES & HUMAN RESOURCES



RESEARCH ADVISORY COMMITTEE

SN	Details of Committee	Designation
1	Prof. H S Subramanya Dean, School of Biosciences Chanakya University, Bengaluru	Chairperson
2	Dr. G. Sivakumar Principal Scientist Division Of Germplasm Conservation and Utilisation, NBAIR, Bengaluru	Member
3	Dr. Sanjay Ghosh Associate Professor, Institute of Bioinformatics and Applied Biotechnology Bengaluru	Member
4	Dr. M. S. Sheshshashayee Professor, Dept. of Crop Physiology, UAS, GKVK, Bengaluru	Member
5	Dr. S. Janarthanan, Professor, Department Of Zoology, University of Madras, Chennai	Member
6	Dr. V. Girish Naik, Retired Scientist-D, Central Silk Board	Member
7	The Director CSR&TI, Mysore	Member
8	The Director CSGRC, Hosur	Member
9	The Director, NSSO, Bengaluru	Member
10	The Director (Tech) Central Silk Board, Bengaluru	Member
11	Scientist-D and Head, Research Coordination Section (RCS), Central Silk Board, Bengaluru	Member
12	The Director Institute for Seri-Biotechnological Research Kodathi, Bangalore	Member- Convener

INSTITUTE BIO SAFETY COMMITTEE

S N	Details of Committee	Remarks
1	Dr. P. J Raju Director, APSSRDI, Hindupur Andhra Pradesh	Chairperson
2	The Director Seri-biotech Research Laboratory, Bangalore	Member convener
3	Prof. Upendra Nongthomba Dept. of Molecular Reproduction and Developmental genetics Indian Institute of Science Bangalore	Member
4	Dr. R. Asokan Principal Scientist Division of Basic Sciences, IIHR, Hesaraghatta Bangalore	DBT Nominee
5	Dr. H.K. Basavaraja Retd. Scientist, Central Silk Board, Bangalore	Member
6	Dr. Raghunath, MBBS Medical Officer, Kodathi, Govt. Medical Hospital, Sarjapura Road, Bangalore	Member
7	Dr. K. M. Ponnuvel Scientist D, Seri-biotech Research Laboratory, Bangalore	Member -Internal Expert
8	Dr. A. R. Pradeep Scientist D, Seri-biotech Research Laboratory, Bangalore	Member -Internal Expert

HUMAN RESOURCES

	Name	Designation
1	Dr. S. Manthira Moorthy	Director
2	Dr. Arun Kumar K P	Scientist- D
3	Dr. G. Subrahmanyam	Scientist- D
4	Dr A. Ramesha	Scientist- D
5	Dr. Pawan Shukla	Scientist- D
6	Dr. K. S. Tulsi Naik	Scientist- D
7	Dr. Rajal Debnath	Scientist- D
8	Dr. Himanshu Dubey	Scientist-C
9	Mr. R. N. Srikantiah	Senior Technical Assistant
10	Mr. Srinivas Raju K.V.	Field Assistant

RESEARCH FELLOWS / ASSISTANTS

	Name	Designation
1.	Ms. Indumathi Kamatchi	Ph.D. Scholar & JRF
2	Ms. Vanitha C.	Ph.D. Scholar & Project Assistant
3	Ms. L. Deepika Iyyangar	Junior Research Fellow
4	Ms. Lavanya C.	Junior Research Fellow
5	Ms. Harshitha Prakash	Junior Research Fellow
6	Ms. Anushree Karna	Junior Research Fellow
7	Mr. Gowtham K	Junior Research Fellow
8	Ms. Roja G	Junior Research Fellow
9	Ms. Shuvangi Das	Junior Research Fellow
10	Ms. Swetha D	Junior Research Fellow
11	Ms. Pooja B N	Junior Research Fellow
12	Ms. Vrinda M	Junior Research Fellow
13	Mr. Babu C V	Junior Research Fellow

ADMINISTRATION

	Name	Designation
1	Ms. Manjula S.	Superintendent
2	Ms. Arul Jyothi	Superintendent
3	Shri Kenchappa	Multi-tasking Staff

PUBLICATIONS

1. Deepika, I., Singh, A., Kumar, I., Gnanasekaran, R., Dubey, H., Debnath, R., Shukla, P., Ponnuvel, K.M., Kumar, V., Arunkumar, K.P. and Gadad, H., Sangannavar P, Moorthy SM, Sivakumar G, Gosh S, Subrahmanya HS, Subrahmanyam G. 2025. Biological and genomic characterization of a cypovirus isolated from golden muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae). *Journal of Invertebrate Pathology*, p.108343.
2. Sivakumar, P, Pandey, S., Ramesha, A. et al. Sporophyte-directed gametogenesis in *Arabidopsis*. *Nat. Plants* 11, 398–409 (2025). *Co-First author
3. Rao, M., Ramesha, A., Dubey, H., Shukla, P., Ponnuvel, K.M., Sivaprasad, V. and Suresh, K (2024). Analysis of Expression, Mutation, and Alternative Splice Variants of Candidate Genes, MLO2 and MLO6A, Involved in Powdery Mildew Susceptibility in Mulberry (*Morus* spp.). *Plant Molecular Biology Reporter*. Vol. 42, pages 784–793.
4. Subrahmanyam, G., Thirupathaiah, Y., Vijay, N., Debnath, R., Arunkumar, K.P., Gadwala, M., Sangannavar, P.A., Manthira Moorthy, S. and Chutia, M., 2024. Contrasting gut bacteriomes unveiled between wild *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae) and domesticated *Bombyx mori* L. (Lepidoptera: Bombycidae) silkworms. *Molecular Biology Reports*, 51(1).
5. Vanitha C., Indumathi Kamatchi Balakrishnan, Rajal Debnath, Lavanya C., Tulsi Naik K.S., S. Manthira Moorthy, Kureeckal V. Ramesh and Himanshu Dubey Genome-wide In-silico Identification of Microsatellites In Eri Silkworm, *Samia ricini* (Lepidoptera: Saturniidae)(2024) *Eco. Env. & Cons.* 30 (4); pp. (435-446)
6. Tulsi Naik, C Vanitha, Sandhya Rasalkar, G Ravi Kumar, Upendra Nongthomba, S Manthira Moorthy Molecular Evidence Supporting the Natural Defense of the Lamerin Breed of Silkworm (*Bombyx mori* L.) to *Nosema bombycis* Infection *Agric Res J* 62 (2): 197-205, April 2025 DOI: 10.5958/2395-146X.2025.00024.
7. Pawan Shukla, Harshitha Prakash, Ramesha A, Himanshu Dubey, S Mahiba Helen, Gangavarapu Subrahmanyam, S Manthira Moorthy and S Gandhi Doss (2024) Identification of honeydew components excreted by pink mealy bug *Maconellicoccus hirsutus* (Green). *Indian Journal of Sericulture (IJS)*. Vol 58, No. 1-2, Issue July-Dec 2024, Page 35-41, ISSN 0445-7722
8. Harshitha Prakash, C Veershetty, Pawan Shukla, G Subrahmanyam, SM Moorthy (2024) Stereo-microscopic study of the anatomy of papaya mealybug *Paracoccus marginatus* (Williams and Granara De Willink). *International Journal of Tropical Insect Science*, 1-10, DOI: 10.1007/s42690-024-01342-1
9. पवन शुकला, रमेशा ए., हिमांशु दूबे एवं एस. मंदिरा मूर्ति (2024) शहतूत पौध में जीनोम एडिटिंग की संभावनाएं published in राष्ट्रीय राजभाषा रेशम तकनीकी सेमिनार on 24.08.2024, ISBN No. 978-81-970698-4-0, page no. 132-134

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