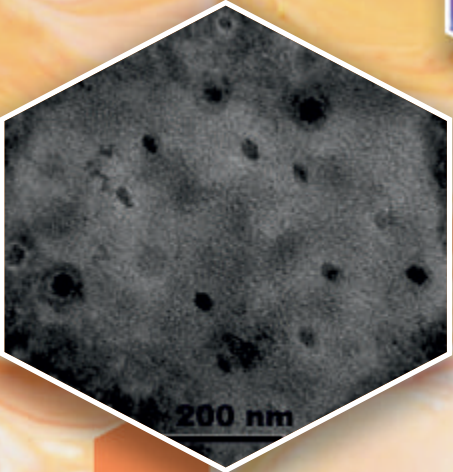
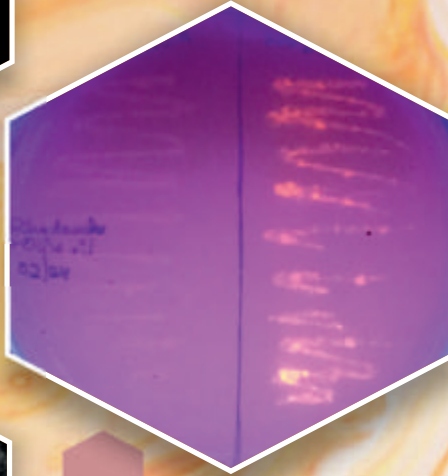


वार्षिक प्रतिवेदन Annual Report

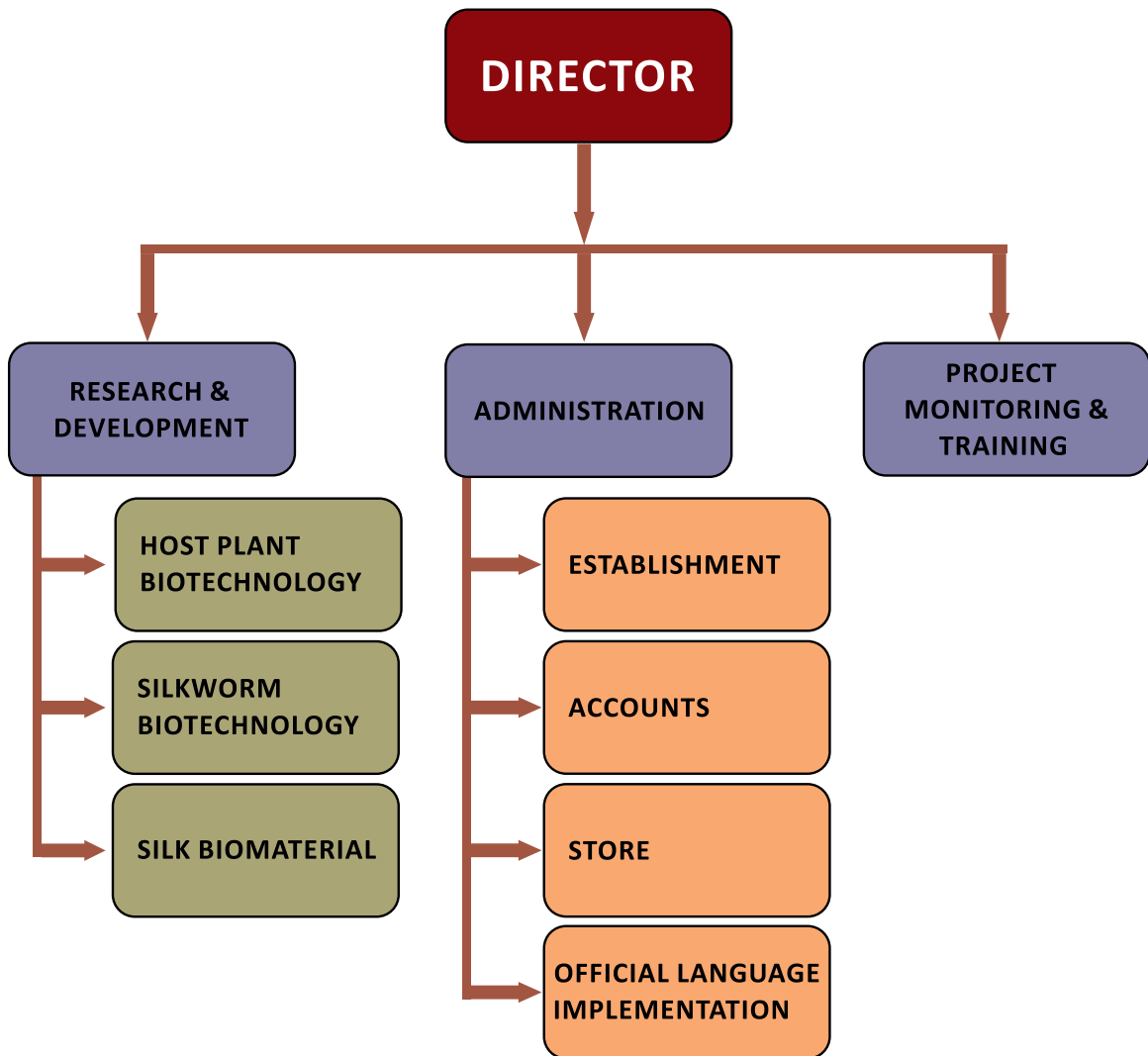
2023-24



रेशम जैव-प्रौद्योगिकी अनुसंधान प्रयोगशाला
SERI-BIOTECH RESEARCH LABORATORY

Central Silk Board
Ministry of Textiles, Govt. of India
Carmelram post, Kodathi, Bengaluru - 560 035

ORGANIZATION CHART







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वार्षिक प्रतिवेदन Annual Report 2023-24



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COVER PAGE

Top most hexagon on cover page represents the lamarin cocoon while the down one is TEM image of AaCPV-4. Centre top image is rhodamine assay plate and below this is lamarin larva. The last hexagon image represents the CSR-2 cocoon.

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प्राक्कथन



मुझे वर्ष 2023-2024 के लिए रेशम-जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला [एसबीआरएल], केंद्रीय रेशम बोर्ड की वार्षिक रिपोर्ट प्रस्तुत करते हुए खुशी हो रही है। वन्य और शहतूती क्षेत्रों में उत्कृष्टता के प्रति हमारी प्रतिबद्धता इस वर्ष किए गए विभिन्न परियोजनाओं में स्पष्ट है।

इस रिपोर्ट में, रेशम के कीड़ों के विभिन्न आणविक पहलुओं पर गहन शोध किया है, जिससे दूरगामी निहितार्थों के साथ उल्लेखनीय सफलताएँ प्राप्त हुई हैं।

उल्लेखनीय उपलब्धियों में साइपोवायरस-4 (AaCPV4) का जीनोम अनुक्रमण शामिल है, जो मूगा रेशमकीट रोगों के जटिल तंत्र को समझने और उनके शमन के लिए नए मार्ग तैयार करने की दिशा में एक महत्वपूर्ण कदम है। साथ ही, BmBDV-प्रतिरोधी डबल हाइब्रिड (FC1RXFC2R) का चिह्नित रेशम कीट पालकों के यहाँ परीक्षण चल रहा है जो कि BmBDV-विषाणु प्रतिरोधी रेशमकीट नस्लों के विकास में एक उल्लेखनीय कदम है।

इसके अलावा, हमारे शोध ने शहतूत के एक कीट, पिंक मीलीबग द्वारा उत्पादित हनीड्यू के संघटन पर पनई जानकारी प्रदान की है। साथ ही, नोसीमा बम्बिसिस से संक्रमित लैमेरिन प्रजाति के रेशम के कीड़ों में संभावित सहनशीलता जीन की पहचान की है। पेबरिन रोग की प्रारंभिक पहचान के लिए एक लेटरल फ्लो अस्से (LFA) किट का विकास हमारे रेशम के कीड़ों के स्वास्थ्य और उत्पादकता में सुधार के प्रति हमारी प्रतिबद्धता का प्रमाण है।

मैं केंद्रीय रेशम बोर्ड के सदस्य सचिव और निदेशक (वित्त) के प्रति उनके अमूल्य सहयोग के लिए आभार व्यक्त करता हूँ। साथ ही, अनुसंधान सलाहकार समिति को उनके मूल्यवान मार्गदर्शन के लिए भी धन्यवाद देता हूँ। इसके अतिरिक्त, मैं डी.बी.टी. जैसी प्रायोजक एजेंसियों को वित्तीय सहायता प्रदान करने के लिए तथा अन्य संस्थानों से प्राप्त हुए सहयोग के लिए आभारी हूँ। अंत में, एसबीआरएल के वैज्ञानिकों एवं शोधार्थियों की भी सराहना करता हूँ जिनकी निष्ठा और योगदान ने हमारी प्रगति को आगे बढ़ाने में महत्वपूर्ण भूमिका निभाई है।

मुझे आशा है कि यह रिपोर्ट हमारी उपलब्धियों का सूचनाप्रद एवं दृष्टिकोणपूर्ण अवलोकन प्रदान करेगी तथा हमारे शोध प्रयासों की निरन्तर वृद्धि और सफलता के लिए आधार तैयार करेगी।।

डॉ. एस. मंदिरा मूर्ति
निदेशक

FOREWORD

It is with immense pleasure that I present the annual report of the Seri-Biotech Research Laboratory (SBRL), Central Silk Board for the year 2023-2024. This document reflects our unwavering dedication to advancing research in both the Vanya and mulberry sectors, showcasing a dynamic range of pioneering projects.

Throughout this year, we have delved deep into the molecular aspects of silkworms, producing notable breakthroughs with far-reaching implications. One of the cornerstone achievements in our research is the genome sequencing of *Antheraea assamensis* cypovirus (AaCPV4), a significant stride towards deciphering the complex mechanisms of muga silkworm diseases and charting new pathways for their mitigation. Additionally, our ongoing field trials involving the BmBDV-resistant double hybrid (FC1RXFC2R) represent a promising milestone in the development of robust and disease-resistant silkworm breeds.

In another key achievement, our investigation into the honeydew composition produced by pink mealybugs, a causative pest of mulberry has provided novel insights, while the identification of potential tolerance genes in Lamarin breed silkworms infected with *Nosema bombycis* underscores our commitment to enhancing silkworm resilience. Furthermore, the development of the Lateral Flow Assay (LFA) kit for the early detection of pebrine disease exemplifies our relentless pursuit of innovations aimed at safeguarding silkworm health and optimizing productivity.

I would like to express my sincere gratitude to the Member Secretary and Director (Finance) of the Central Silk Board for their invaluable support. My heartfelt thanks also go to the Research Advisory Committee for their insightful guidance. Additionally, I extend my appreciation to our esteemed funding partners, such as DBT, and to our collaborative institutions, whose support has been indispensable. Finally, I would like to acknowledge the innovative spirit of the SBRL scientists and research scholar, whose dedication and hard work have been instrumental in propelling our progress forward.

It is my hope that this report serves as both an informative and thought-provoking overview of our accomplishments and sets the stage for the continued growth and success of our research endeavors.



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

MANDATE

- 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.
- To collaborate with national and international organizations for strengthening the basic and applied research in seri-biotechnological aspects.
- To disseminate and commercialize the tools & technologies among the stakeholders.
- To develop quality human resources in the area of seri-biotechnology.

अनुसंधान के मुख्य आकर्षण

1. एंथेरिया एसामेंसिस सिपोवायरस-4 (AaCPV-4) का जीनोम अनुक्रमण किया गया।
2. वन्य रेशमकीटों में पेब्राइन रोग का पता लगाने के लिए लेटरल फ्लो एसे (एलएफए) तकनीक का विकास किया गया।
3. मूगा रेशमकीट, एंथेरिया एसामेंसिस हेल्पर में अटैसिन जीन फैमिली का विश्लेषण किया गया।
4. शहतूत में टुकरा रोग का एक कारक कीट, पिंक मीलीबग के हनीड्यू का रासायनिक संघटन पता किया गया।
5. लैमेरिन प्रजाति में नोज़ेमा बॉम्बिसिस संक्रमण के खिलाफ सहिष्णु/प्रतिरोधी जीनों की पहचान और सत्यापन किया गया।
6. BmBDV विषाणु प्रतिरोधी प्रजातियों के प्रदर्शन का मूल्यांकन OST और OFT के माध्यम से

RESEARCH HIGHLIGHTS

1. Deciphered the genome sequence of *Antheraea assamensis* cypovirus-4 (AaCPV-4)
2. Developed of Lateral Flow Assay (LFA) technology for the detection of pebrine disease in Vanya silkworms.
3. Analysed of Attacin gene family in Muga silkworm, *Antheraea assamensis* Helfer
4. Identified honeydew composition of pink mealybug. *Maconellicoccus hirsutus* (Green), a causative pest of tukra disease in mulberry.
5. Identified and validated tolerant /resistant genes in response to *Nosema bombycis* infection in lamerin breed.
6. Evaluated performance of BmBDV resistant bivoltine silkworm hybrid through On-station and On-farm Trial.

LIST OF RESEARCH PROJECTS

ON-GOING RESEARCH PROJECTS

1. **ARP-08007MI:** Biological and molecular characterization of virosis in Muga silkworm, (*Antheraea assamensis* Helfer) (March 2022- February 2025)
2. **AIB-08008MI:** Development and Evaluation of Eri silkworm (*Samia ricini* Donovan) breeds/hybrids with improved productivity (March 2022- February 2025)
3. **AIC-8009CN:** Profiling of lipid, protein and carbohydrate of mulberry mealybug *Maconellicoccus hirsutus* (Green) for its control (April 2022- March 2025)
4. **AIT-08010MI:** Transcriptome studies for susceptibility of Muga silkworm (*Antheraea assamensis* Helfer) to cypovirus (April 2022- March 2026)
5. **AIE08011MI:** Evaluation of NPV tolerant bivoltine hybrids and cross breeds at farmer's level (May 2022 to June 2024)
6. **SRP08012MNC:** Development of a Rapid Antigen Test Kit for Diagnosis of Cytoplasmic Polyhedrosis in Vanay Silkworms (*Antheraea assamensis*, *A. mylitta* and *A. proylei*) (Feb 2024 to January 2026)
7. **SIT08014MIC:** Development of molecular barcodes for commercially important silkworm (*Bombyx mori*) breeds/hybrids (March 2024 to Feb 2026)
8. **SIT08015MIC:** Validation of SNP markers associated with economic traits in *Bombyx mori* L. (March 2024 to Feb 2026)
9. **PRP08013MIC:** Mapping and Evaluation of Mulberry Rootstock for Root rot (*Lasiodiplodiatheo bromae*) Resistance (March 2024 to Feb 2027)

CONCLUDED RESEARCH PROJECTS

1. **AIT-08003CN:** Gene expression profiling for the identification of resistant / tolerant genes to microsporidian infection in Lamerin breed of silkworm, *Bombyx mori* L. (August 2019 - August 2023); **DBT Funded**
2. **AIT-08006 EF:** Development of lateral flow assay (LFA) kit for diagnosis of pebrine disease in silkworms (March 2021 - January 2023), **DBT-BIRAC Funded**

PROGRESS OF ONGOING RESEARCH PROJECTS

1. ARP08007MI: Biological and molecular characterization of virosis in Muga silkworm (*Antheraea assamensis* Helfer)

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

G. Subrahmanyam, K.M. Ponnuvel (Up to May 2023), K. Indira Kumar*, Abhishek Singh[#]

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

[#]P3 Unit, CSB-Muga Eri Silkworm Seed Organization (CSB-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

Virosis is a dreadful disease in Muga silkworm and an average of 40% crop loss normally occurred due to incidence of disease. The current project is aimed to identify the viral pathogen, to establish epidemiology of virosis disease and to characterize the viral pathogen in Muga silkworm through biological and genome sequence analysis. Further, the viral pathogenesis, tissue tropism, mode of transmission and source of infection was investigated so as to develop an appropriate molecular based diagnostic technique for early detection of the virus.

TEM Study

The purified viral polyhedra/ OB sample was pelleted by centrifugation at 5000g for 5 min, resuspended with 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.2) and fixed overnight at room temperature. Then the samples were dehydrated with series of ethanol solutions and stained for 1 h with 2% uranyl acetate. The samples were further embedded in araldite resin. After polymerization, samples were ultrathin sectioned, transferred to TEM grids, and stained with lead acetate. The resulting grids were observed under a transmission electron microscope.

Viral genome sequencing and analysis

The good quality RNA obtained from the CPV polyhedral bodies was utilized for library preparation using the kit NEBNext Ultra II Directional RNA Library Prep Kit for Illumina. The library was sequenced on Illumina platform, Nova Seq 6000 (Illumina, San Diego, USA). The raw reads were processed through FastQC (Andrews, 2010) and Trimmomatic tools (Bolger et al., 2014) to remove adapter contamination and low-quality bases. Good quality pre-processed reads were utilized for *de-novo* assembly using Trinity-v2.15.1 (Grabherr et al., 2011). The contigs obtained from the *de-novo* assembly were translated using TransDecoder tool (<https://github.com/TransDecoder/TransDecoder>) to predict ORFs and subsequent comparison with closely related CPV strains.

The phylogenetic information of the *A. assamensis* CPV was deciphered through RNA dependent RNA polymerase (RdRp) gene using MEGA version 11.0 (<https://www.megasoftware.net/>). The deduced amino acid sequences of RdRp genes from different lepidopteran cypoviruses were retrieved from the GenBank-NCBI database and these sequences were aligned to infer phylogeny of *A. assamensis* cypovirus. Phylogenetic relationships among different lepidopteran cypoviruses were inferred using the maximum likelihood method available in the MEGA version 11.0 (<https://www.megasoftware.net/>).

Results:

The TEM image showed the presence of occlusion derived viruses (ODVs). The spherical virions were embedded in a single polyhedral matrix. This confirms that the virus purified from infected *A. assamensis* silkworms were ODVs (**Fig. 1**). The size of the virus particles was approximately 60 nm.

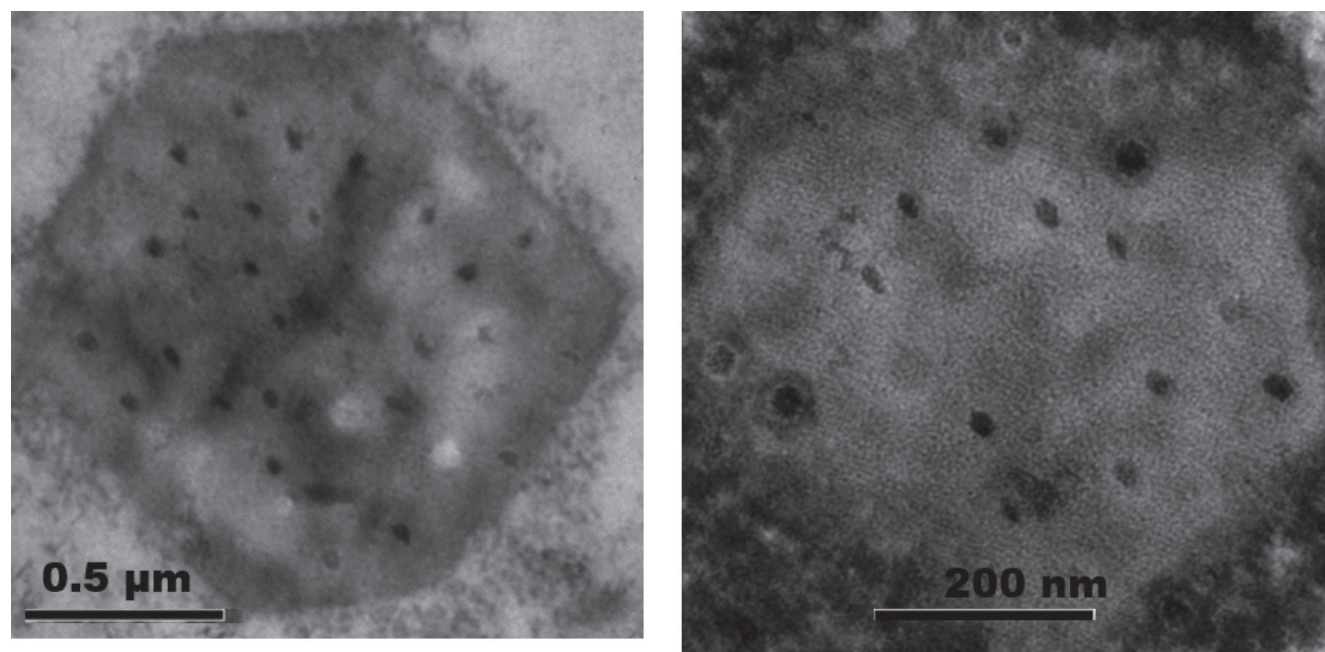


Figure 1: TEM image of AaCPV-4

Viral genome sequencing and analysis

The genome size was estimated to be 24.9 kb. The genome of AaCPV4 consists of 10 discrete segments. All the 10 ORFs were defined and structural features were assigned. The longest segment was found to be segment 1 with 3.85 kb in size encoding minor capsid protein whereas the smallest segment was found to be segment-10 with 1.1 kb in size encoding polyhedrin. Each segment contained a unique open reading frame with conserved non-coding regions (NCR) that featured at both the 5' and 3' termini. The NCR region at 5' end was identified as "AGTAAT" whereas the NCR region at 3' termini was found to be ATAGAGC except in segment 9. The evolutionary history of AaCPV-4 was inferred by using the

Maximum Likelihood method and Le_Gascuel_2008 model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (**Fig. 2**). The evolutionary lineage for both Cypovirus 1 and 4 (cluster-1) was found to be the same and shared a common ancestor with significant bootstrap value (>90%). Similarly, the evolutionary lineage of Cypovirus 4 and 22 (cluster-3) was found to be the same with shared a common ancestor.

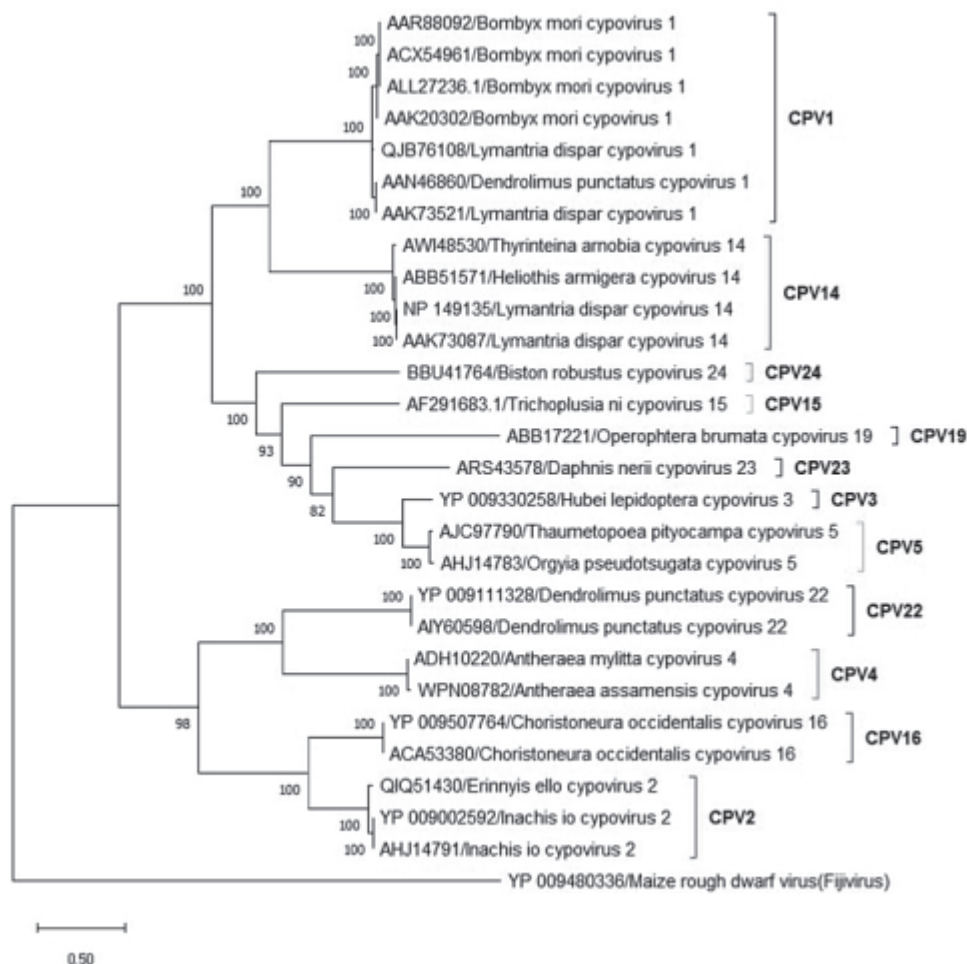


Figure 2: Phylogenetic relationships between cypovirus isolates using the deduced amino acid sequences of the RdRp protein. Bootstrapping values (1000 replicates) above 80 % are shown.

2. AIB08007MI: Development and evaluation of eri silkworm (*Samia ricini* Donovan) breeds / hybrids with improved productivity

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

Rajal Debnath, Ramesha A, Himanshu Dubey, Prateesh Kumar PM¹,
Lalitha Natarajan² & Malashree N³, S. Manthira Moorthy
¹CSB-ESSO-Hosur, ²P2-BSF-Topatoli, ³CSB-SSTL, Kodathi

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 naturally comprise four distinct phenotypes, differentiated by body color and the presence or absence of black markings: YP (yellow plain), GBP (greenish blue plain), YZ (yellow zebra), and GBZ (greenish blue zebra). These morphotypes coexist within the same population depending upon the ecotypes, and it is hypothesized that the silk produced by each morphotype may differ in content and yield.

Since, the mixed nature of the populations makes it difficult to accurately assess the genetic potential and silk production capacity of each individual morphotype, we developed sib-mated lines of each of the four morphotype to create non-segregating (homozygous) lines (for body color and marking phenotype) that allowed the isolation of genetic traits specific to each phenotype. By removing the genetic variability present in mixed populations, our study assessed the silk yield and quality associated with each morphotype. This approach provided a controlled setting to explore whether these phenotypes correlated with variation in silk yield and production, contributing to the understanding of the genetic factors underlying silk yield.

A correlation was observed between silkworms with larval markings and increased cocoon/pupal weight in our non-segregating stable lines (generation 11) compared to lines devoid of any markings. The mean from the distribution of cocoon weights from YZ and GZ stable lines were significantly higher than that of plain lines (YP & GP) ($p < 0.05$) (**Fig. 3**).

The observed correlation between the presence of larval markings and increased weight in our non-segregating stable lines suggests that the genetic factors responsible for the marking phenotype may be indirectly influencing these traits. If these genes are inherited together, marked individuals could consistently produce more silk, leading to heavier cocoons.

A genetic crossing experiment with the stabilized lines suggested greenish body color was dominant over yellow body color (**Fig. 4**). The progeny proportions did not account for the marking phenotype but only on the body color phenotype. ddRADseq genotyping of the stabilized lines is being undertaken to gain further insights into the developed lines. The stable lines are currently selected and reared for the traits mentioned above.

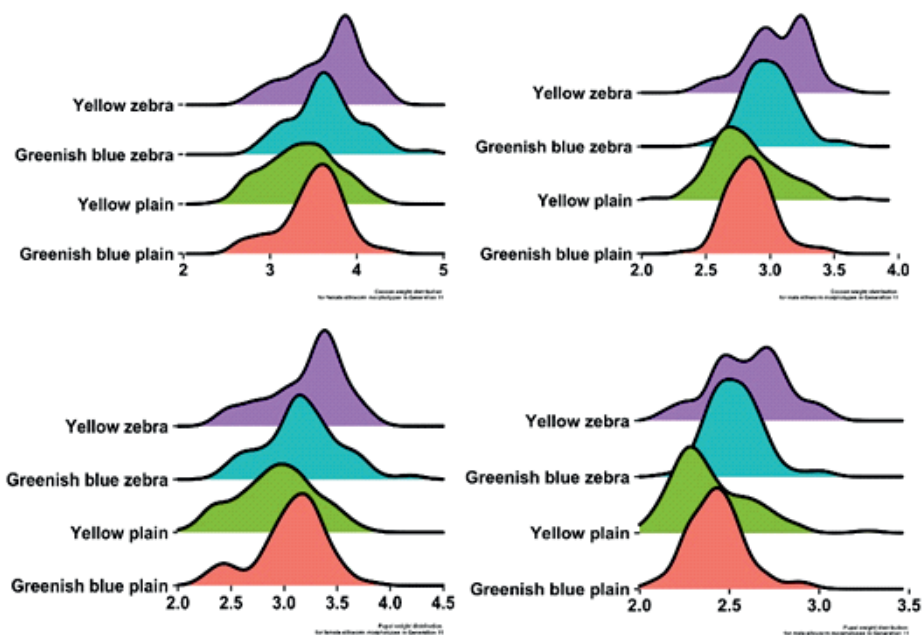


Figure 3: Distribution of cocoon and pupal weight of the fixed non-segregating stable morphotypic lines at G11 generation. The median weights of both cocoon and pupa for zebra lines in both yellow body colour and greenish blue were higher than plain morphotypes ($p < 0.05$)

♀	♂	Cross	Progeny proportions		Gg or GG	
			yellow %	greenish blue %	gg	gG or GG
		YP X GBP	55.0 %	45.0 %		
		YP X YZ	100 %	0		
		YP X GBZ	0.7 %	99.3 %		
		GBP X YP	15.8 %	84.2 %		
		GBP X YZ	0	100 %		
		GBP X GBZ	0	100 %		
		YZ X YP	100 %	0		
		YZ X GBP	0	100 %		
		YZ X GBZ	0	100 %		
		GBZ X YP	44.8 %	55.2 %		
		GBZ X GBP	0	100 %		
		GBZ X YZ	29.6 %	70.4 %		

Figure 4: 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.

3. AIC08009CN: Profiling of lipid, protein and carbohydrate of mulberry mealybug *Maconellicoccus hirsutus* (Green) for its control

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

Pawan Shukla, Ramesha A, Himanshu Dubey, Mahiba Helen¹, Kesavan Subaharan²

¹CSB-CSR&TI, Mysore, ²ICAR - National Bureau of Agricultural Insect Resources, Bangalore

Objective:

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

Pink mealybug (*Maconellicoccus hirsutus*) is one of the significant mulberry pests that damages the mulberry crop at shoot tips causing bunchy top also known as 'tukra disease' affecting the silkworm rearing in which, mulberry silkworm (*Bombyx mori*) being a monophagous is solely dependent on the mulberry leaves which in turn affects the quality silk production. Mealybugs are soft bodied, which continuously sucks the phloem sap of host plants. While sucking the sap it releases toxic substances into the plants which cause tukra disease where in the leaves become thick, dark and curls at the apical part of the plant. In order to protect themselves from the external environment they secrete wax around its body, it also excretes sugary liquid known as honeydew which causes sooty moulds a secondary infection, attracting the ants and maintains a mutualistic relationship.

One of the notable characters of pink mealybugs is secretion of a sticky, sugary substance known as honeydew. The specific composition can vary depending on factors such as the insect species, the host plant, and environmental conditions. In the current study we have collected honeydew of pink mealybug and stored under hexane (**Fig. 5A**). To prepare the honeydew samples for GC-MS analysis, they were derivatized through hydrolysis and trimethylsilylation. The 1 μ L was injected into the GC-MS for analysis. The column used in GC-MS was DB-5MS. Samples were injected in a split mode with a 1: 5 dilutions i.e. 2 μ L in 20 μ L of hexane and the flow through the column was held constant using helium as carrier gas at 1ml/min. The initial oven temperature was set at 60 $^{\circ}$ C for 1 min. thereafter, the temperature was raised with a gradient of 20 $^{\circ}$ C per min to 190 $^{\circ}$ C and held for 8.5 min. Afterward, the temperature was raised with a gradient of 5 $^{\circ}$ C per min to 280 $^{\circ}$ C for 29.5 min. The data were analysed in Mass Hunter software.

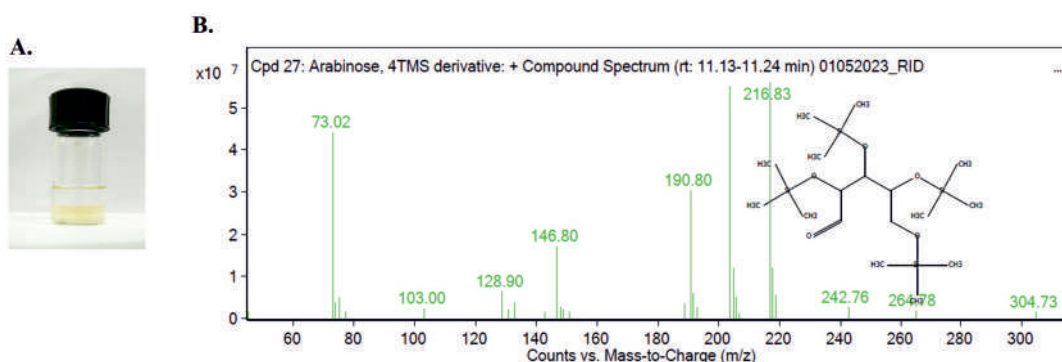
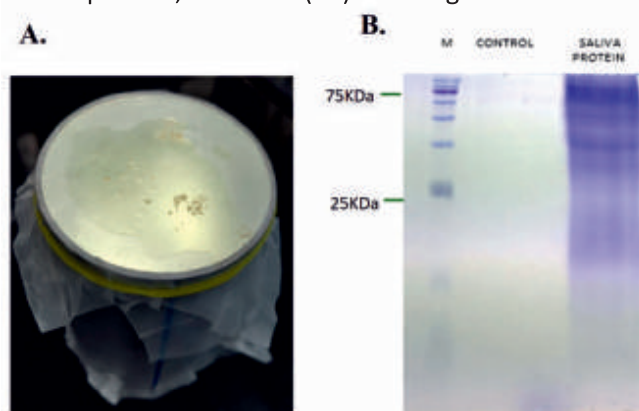


Figure 5: Collected Honeydew (**A**) and representative image of mass spectrum of honeydew of pink mealybug through GC-MS (**B**)

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- ChiroInositol	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

The GC-MS data analysis revealed that the honeydew of pink mealybug contains a complex mixture of compounds which include sugars, sugar alcohols, organic compounds and organic acids viz., Arabinose, D-Glucopyranose, D-Mannose, Gluconolactone (3R,4S,5R,6R), Glycerol, Lactic acid, Levoglucosan, Levulinic acid, Mannonic acid Lactone, Myo-inositol, and Pentanoic acid (**Fig. 5B, Table 1**). Out of which four (04) were monosaccharides, five (05) organic compounds, and three (03) were sugar alcohols.

**Figure 6:** Artificial diet with pink mealybug crawlers (A) and SDS PAGE for saliva protein (B)

Pink mealybug while sucking the sap, releases toxin substances to the host plant which causes disease to the plant. Saliva from pink mealybug crawlers were collected using non-destructive methods (rearing it on artificial diet), followed by dialysis, protein precipitation and SDS-PAGE (Fig. 6A & B).

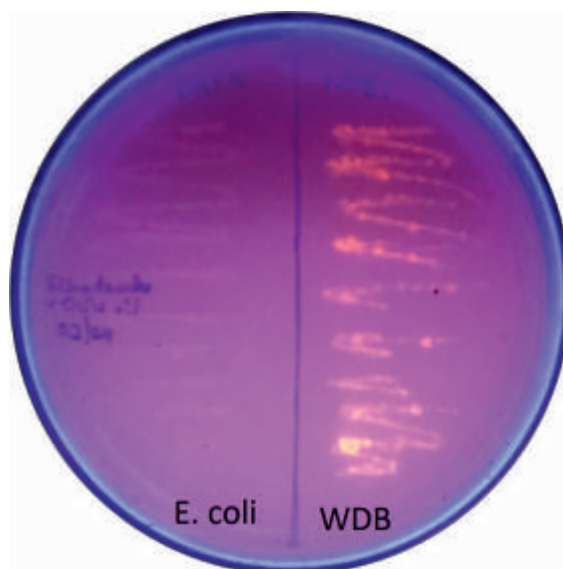


Figure 7: Rhodamine assay for wax degradation in which *E. coli* used as control. Under UV light, Rhodamine B dye binds to free fatty acids released via wax degradation and glows as yellow/orange color.

With the aim to degrade mealybug wax we have identified the wax degrading bacteria from soil. Serially diluted soil samples were spread plated on minimal agar media along with mealybug wax. Three colonies were selected from the grown plates and streaked on luria Bertani agar and made glycerol stock for further use. Later performed IMViC test for all 3 different strains. Rhodamine test was done also performed to confirm the wax degradation property of wax degrading bacteria (WDB)(Fig. 7)

4. AIT08010MI: Transcriptome studies for susceptibility of Muga silkworm (*Antheraea assamensis* Helfer) to cypovirus

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

Himanshu Dubey, Rajal Debnath, G. Subrahmanyam, Vikram Kumar¹, & Abhishek Singh²

¹P3 unit of CSB-MESSO Rompara, Meghalaya; ²P3 Unit of CSB-MESSO Nongpoh, Meghalaya

Objective:

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.

The Muga silkworm (*Antheraea assamensis* Helfer), a wild species indigenous to the northeastern states of India, including Assam, Meghalaya, and Arunachal Pradesh. This silkworm can undergo up to six rearing cycles annually, including commercial, pre-seed, and seed crops, relying on host plants such as Som (*Persea bombycina*) and Soalu (*Litsaea monopetala*) in outdoor conditions. The present study focuses on investigating the molecular response of Muga silkworms to cypovirus infection, with the aim of elucidating the molecular changes during host-pathogen interactions. Initially, the project's objective

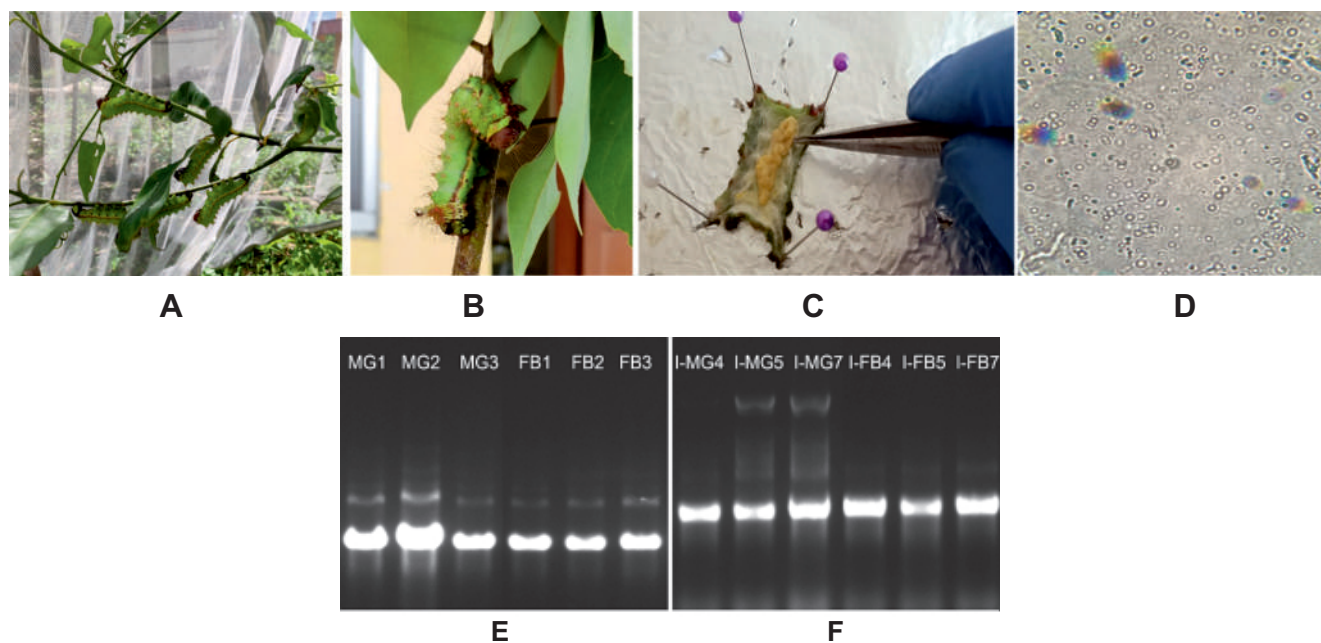


Figure 8: Inoculation of Muga silkworm with pathogen and RNA isolation from different tissues. (A) Control silkworm (B) Pathogen inoculated silkworm (C) Dissection (D) CPV polyhedral bodies in pathogen inoculated silkworms (E & F) Agarose Gel electrophoresis of RNA isolated from control and pathogen challenged Muga silkworm.

was to assess how seasonal environmental variations influence the susceptibility of Muga silkworms to various pathogens using a transcriptomic approach. However, following recommendations from the Research Advisory Committee of SBRL, the focus was refined to study a specific pathogen responsible for significant crop losses in the Muga silk industry. The project was thus redirected to explore the immune response of Muga silkworms to cypovirus infection, a viral pathogen known to cause virosis disease. Consequently, the project title and objectives were modified, and the research work initiated accordingly. Muga silkworms were experimentally challenged with cypovirus, and tissue samples were collected for transcriptome and miRNA analysis. RNA was extracted from the collected samples, and its integrity and concentration were evaluated using agarose gel electrophoresis and Nanodrop spectrophotometry. The figure depicted below shows the control and pathogen challenged silkworms, further microscopy was employed to confirm the presence of cypovirus polyhedral bodies in pathogen-challenged samples (**Fig. 8, Table 2**).

Table 2: Quantification of RNA samples isolated from different tissues

Sample	Concentration (ng/μl)	260/280	260/230
MG1	1709.4	2.1	1.52
MG2	1870.8	2.14	2.03
MG3	1708.5	2.11	1.77
FB1	1074.8	2.11	1.06
FB2	946.7	2.17	1.5
FB3	1041.1	2.18	2.19
I-MG4	1566.5	2.16	2.22
I-MG5	1174.8	2.15	2.16
I-MG7	1632.5	2.16	2.13
I-FB4	983.5	2.15	2.08
I-FB5	1210.5	2.15	2.07
I-FB7	547.7	2.10	1.35

5. AIE08011MI: Evaluation of NPV tolerant bivoltine hybrids and cross breeds at farmer's level

(Collaboration with CSB-CSR&TI Berhampore; Duration: May 2022 to June 2024)

G. Subrahmanyam, K.M. Ponnuvel (Up to May 2023), Tulsi Naik, AR. Pradeep¹

¹CSB-CSR&TI Berhampore

Objective:

1. Evaluation of NPV tolerant bivoltine hybrid, MASN4 x CSR4 at farmer's level in North and North-West regions
2. Evaluation of NPV tolerant bivoltine hybrid, MASN4 x CSR4 at farmer's level in North and North-West regions

B. mori larvae are highly susceptible to different viral diseases among which Grasserie caused by BmNPV causes approximately 25% crop loss in sericulture of different countries. Therefore, developing a silkworm race with better pathogen tolerance and high yield traits is necessary to increase the silk production. NPV tolerant bivoltine and cross breeds were developed through introgression of DNA markers associated with NPV resistance using Marker Assisted Selection Breeding under the project ARP 3605. Three genic markers such as protein kinase G-1B (Nag65), glucosyl transferase (Nag 84) and deoxy kinase (Nag 88) (associated with NPV tolerance) were used for marker assisted selection (MAS) and breeding programs through five back cross generations. The hybrids developed from MASN lines (Nistari x MASN4 and MASN4 x CSR4) are potential and suitable for North and North-West India. The performance of Bivoltine MASN 4 x CSR4 hybrids in respect of yield and cocoon quality is better than ruling hybrids (SK6 x SK7) in West Bengal and at par or better at Himachal Pradesh and Jammu than ruling hybrid FC1 x FC2. Therefore, the MASN hybrids(Nistari x MASN4 and MASN4 x CSR4) are to be race authorized at farmers level in West Bengal and north- west and North-esat India against the existing ruling hybrids.

MASN lines were maintained through continues rearing and the cocoon parameters were mentioned in **table 3**. A total of 92750 nos of MASN4xCSR4 (bivoltine hybrid) DFLs and 25650 DFLs of Nistarix MASN4 (Cross breed) were tested in East and North-West during 2018-2020 in a concluded proejct ARP 3605. In the current project, reaming 40000 DFLs of Nistari x MASN4 DFLs were tested at locations distributed in WB and Tripura (**Fig. 9, 10 and 11**). The cocoon weights for Nistari x MASN (n = 40 each) were found to be Cocoon weight: 1.179±0.106 g (male) 1.566±0.102 (female). Further, the Shell (silk) ratio: 18.2 % (male) 15.5 % (female) were found.

Table 3: Cocoon characters of MASN silkworm lines

Sl. no.		MASN6			MASN4			MASN7		
		Shell wt. (g)	Cocoon wt. (g)	SR (%)	Shell wt. (g)	Cocoon wt. (g)	SR (%)	Shell wt. (g)	Cocoon wt. (g)	SR (%)
1	Females	0.36	1.33	27.07	0.37	1.5	24.67	0.35	1.77	19.77
2		0.24	1.16	20.69	0.31	1.66	18.67	0.39	1.92	20.31
3		0.28	1.46	19.18	0.3	1.57	19.11	0.36	1.76	20.45
4		0.32	1.43	22.38	0.32	1.65	19.39	0.34	1.56	21.79
5		0.3	1.52	19.74	0.28	1.49	18.79	0.39	1.75	22.29
6	Males	0.3	1.63	18.40	0.35	1.52	23.03	0.29	1.25	23.20
7		0.34	1.82	18.68	0.31	1.35	22.96	0.32	1.49	21.48
8		0.35	1.79	19.55	0.3	1.35	22.22	0.36	1.48	24.32
9		0.35	1.52	23.03	0.32	1.4	22.86	0.29	1.18	24.58
10		0.32	1.72	18.60	0.43	1.64	26.22	0.32	1.28	25.00
		Avrg. SR% of MASN6		20.73 (2.65)	Avrg. SR% of MASN4		21.79 (2.59)	Avrg. SR% of MASN7		22.32 (1.83)


Figure 9: Cocoon characters of Nistari X MASN 4



NxMASN-4 FIFLD TRIAL IN MALDA



Figure 10: OFT trails at Malda

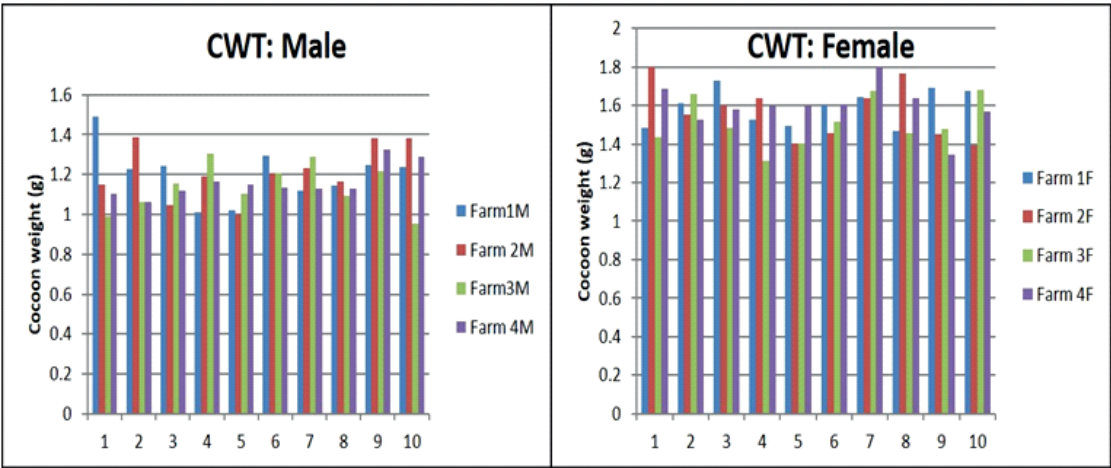


Figure 11: Cocoon weight parameters of male and female cocoons of Nistari X MASN 4

Outcome and utility of the project: Cross breeds of the MASN lines were developed for improved BmNPV tolerance. The breed, Nistari x MASN4 showed better performance in for Bm-NPV tolerance in North-West India.

6. SRP08012MNC: Development of a Rapid Antigen Test Kit for Diagnosis of Cytoplasmic Polyhedrosis in Vanay Silkworms (*Antheraea assamensis*, *A. mylitta* and *A. proylei*)

(In collaboration with CSB-CMER&TI Lahdoigarh and CSB-CTR&TI, Ranchi;

Duration: February 2024 to January 2026)

G. Subrahmanyam, Rajal Debnath, HS. Gadad¹, Brunda²

¹CSB-CTR&TI Ranchi; ²CSB-CMER&TI, Lahdoigarh

Objective:

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 silk industry the i faces significant challenges, primarily stemming from the outbreak of various diseases such as virosis, pebrine, flacherie, and muscardine. Among these ailments, virosis is a major issue in summer seed crop seasons, leading to an average Muga crop loss of approximately 40% to 100%. Additionally, other wild silkworm species, including *A. mylitta* and *A. proylei*, experience substantial mortality rates, with crop losses exceeding >50%. This high susceptibility to diseases, particularly virosis, acts as a major impediment to the developmental progress of Vanya sericulture (Muga and Tasar sector) in India. Under the ARP08007MI project, we have recently made strides in understanding the causative pathogen and epidemiology of virosis in Muga culture. These findings are crucial for developing strategies to mitigate the impact of diseases on the Muga silk industry and contribute to the sustainable growth of Vanya sericulture in the region.

A double stranded segmented RNA virus viz. Cypovirus 4 (Reoviridae) is found to be causative pathogen of virosis disease in *A. assamensis*, *A. mylitta* and *A. proylei*. The virosis disease is now renamed as Cytoplasmic Polyhedrosis. The mortality due to this viral infection predominantly occurs in the late instars during summer crops (June- September) and till date no effective detection methods and control measures have been successfully employed. The current project was recently implemented (February 2024) to develop point of acre diagnostics and prophylactic measures to control Cytoplasmic Polyhedrosis in vanya sericulture. The viral pathogen is sequenced and structural and non-structural proteins were analysed. Recombination and genome reassortment are common in segmented ds-RNA viruses. Nevertheless, our initial studies could not find any recombination events among similar Cypovirus-4 strains (**Fig. 12**)

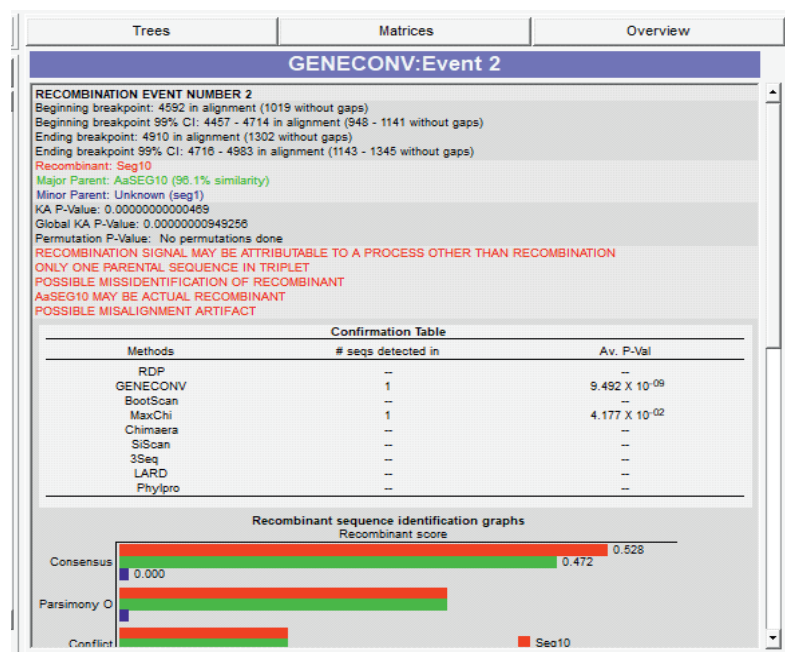
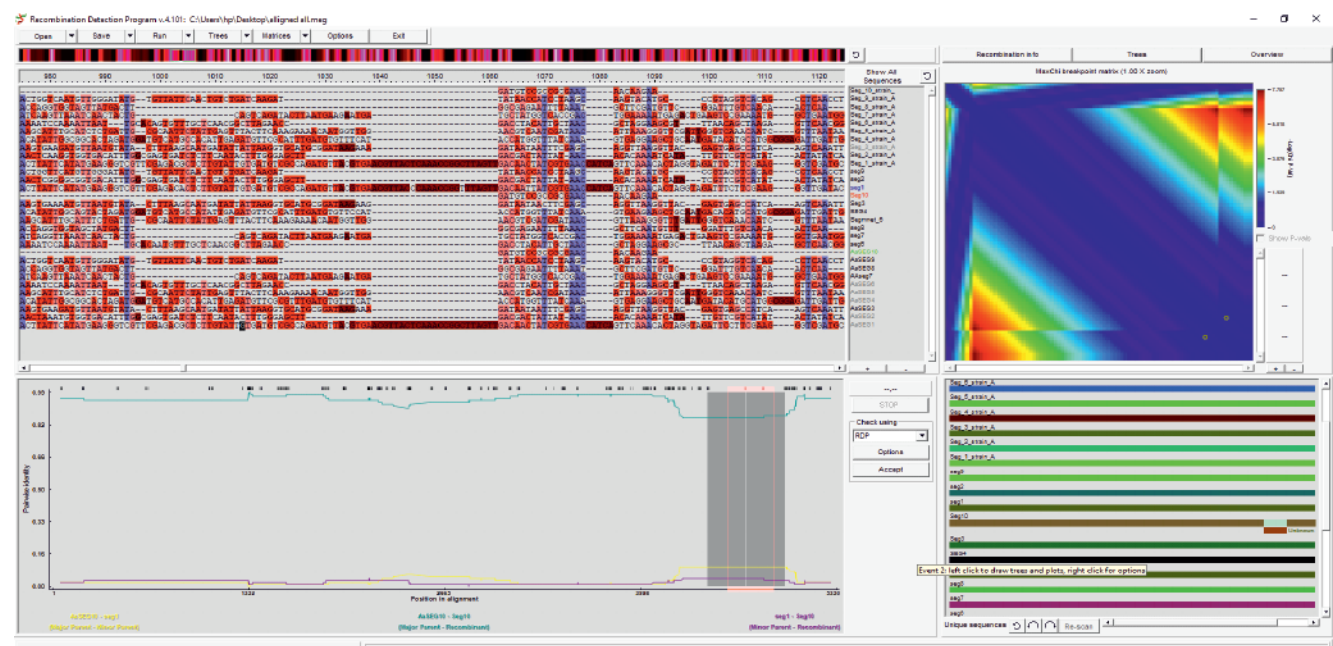


Figure 12: Maxchi recombination breakpoint analysis of *Aacpv4* genomes



7. SIT08014MIC: Development of molecular barcodes for commercially important silkworm (*Bombyx mori*) breeds/hybrids

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

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

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

Objective:

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.

Molecular barcoding is a powerful tool used for authenticating breeds/hybrids in different sectors such as agriculture and animal husbandry. It also facilitates conservation efforts, genetic improvement, and intellectual property protection. The domestic silkworm *Bombyx mori* holds substantial economic importance in India due to its role in silk production, rural employment, export revenue, and cultural significance. Although other countries have advanced the genomic characterization of silkworms, India requires barcoding for key commercial hybrids, including FC1 × FC2, and SK6 × SK7. Developing and implementing molecular barcodes for *B. mori* is essential for quality control, safeguarding IP, and ensuring genetic purity, particularly with the anticipated entry of private seed producers into the silkworm seed sector. Developing barcodes for the economically important breeds/hybrids is imperative to support the sustainable growth of the sericulture industry.

8. SIT08015MIC: Validation of SNP markers associated with economic traits in *Bombyx mori* L.

(In collaboration with CSB-CSR&TI, Mysore and P3 Farm, CSB-NSSO, Mysore;

Duration: March 2024 to February 2026)

Himanshu Dubey, Rajal Debnath, Kusuma L¹, N. Chandrakant¹, K. M. Ponnuvel (Up to May 2023)²

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

Objective:

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

The present study is based on the outcomes of the previous DBT-sponsored project, AIT3628 – “Assessment of SNP Variation in Silkworm (*Bombyx mori* L.) by Genotyping by Sequencing and Genome-Wide Association Mapping of Important Commercial Traits”, which concluded in March 2021. In that study, five SNPs were identified: WC-051360.1 associated with yield, WC-051365.1 with thermo-tolerance, NW-021618427.1 with cocoon weight, WC-051360.1 with shell weight, and NW-023678039.1 with filament length, indicating significant associations with corresponding economic traits. However, these findings could not be validated in the earlier project. Therefore, the present study aims to validate these SNP-trait associations to augment their robustness and applicability in future breeding programs.

9. PRP08013MIC-Mapping and Evaluation of Mulberry Rootstock for Root rot (*Lasiodiplodia theobromae*) Resistance

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

Ramesha, A, Pawan Shukla, Himanshu Dubey, Arunakumar G. S.¹

¹CSB-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 disease is a major soil-borne disease in mulberry cultivation in Southern India and which seriously affects leaf yield, and quality for silk production. To develop mulberry varieties for biotic and abiotic stresses, large number of segregation population need to be screened. The phenotyping may not be effective for large number of population and it is dependent on many variable factors such as environment, inoculum, treatment etc. Therefore, identifying and deploying molecular markers for biotic and abiotic stresses increases efficiency of breeding.

Aim of mulberry breeding is to deploy as many desirable traits possible in one variety. However, it is difficult due to only two parents are involved in hybridization and those parents may not carry many desirable traits for both above ground and below ground traits. But grafting breakdowns one problem into two easier to solve problems with different objectives. One objective is to develop above ground traits or shoot (Scion) and another one improvement of root system (rootstock). Both scion and rootstock can be joined by grafting to obtain variety for improved traits for both shoot and root characters. Grafting has increased the efficiency of crop breeding by selecting root and shoot traits independently instead of requiring both sets of traits present in a single individual.

The aim of this project is to map the genomic regions or identifying genes responsible for root rot resistance phenotype. And to develop rootstock-scion combinations to combat root rot resistance with good yield traits through grafting approach for commercial cultivation.

OUTCOME OF CONCLUDED RESEARCH PROJECTS

1. AIT08003CN: Gene Expression Profiling for the Identification of Resistant/Tolerant Genes to Microsporidian Infection in Lamerin Breed of Silkworm, *Bombyx mori* L.

(In collaboration with IISc, Bangalore; Duration: August 2019 to Aug 2023)

Ravi Kumar G. (Up to June 2020)¹, Tulsi Naik K S, Upendra Nongthomba², K. Vijayan (Up to May 2021)³

¹CSB-CSGRC, Hosur; ²IISc, Bangalore, ³CSB, Bangalore

Objectives:

1. Transcriptional analysis of Microsporidian resistant / tolerant and susceptible silkworm breed
2. Identification of Genes responsible for combating microsporidian infection.
3. Identification of miRNAs in response to microsporidian infection.
4. Expression analysis of selected genes/miRNAs.
5. Functional Characterization of selected genes.

The silkworm, *Bombyx mori*, has been domesticated for >5000 years. Today India is the second-largest producer of silk in the world next only to China producing 34,923 metric tons of raw silk annually (CSB note on sericulture 2021-2022). The silkworm, *B. mori* and related species are susceptible to different kinds of diseases caused by pathogens. The most common pathogens infecting them are nucleopolyhedrovirus (NPV) and densovirus, infectious flacherie virus (IFV), cytoplasmic polyhedrovirus (CPV), microsporidians particularly *Nosema bombycis*. Among all pathogens, *Nosema bombycis* causing “Pebrine disease” is responsible for the significant economic loss in the sericulture industry (Bhat et al., 2009). It was one of the factors contributing to the decline of the silk industry in France during 1856 (Li et al., 2017)

Microsporidia are intracellular fungal parasites of all major animal lineages, apart from causing human infections in immuno-compromised patients, microsporidia also cause extensive damage to aquaculture and apiculture industry. Microsporidiosis has been a focus of research for over 150 years and was even the subject of study of Louis Pasteur who investigated the cause of the Pebrine disease of silkworms that threatened to devastate the silk industry in the 19th century. The *N. bombycis*, is the first microsporidian to be recognized, by Nageli in 1857 and still remains a threat to silk industry because of its unique and recurrent occurrence and is the only silkworm disease transmitted vertically through mother moth to offspring and horizontally. The disease spreads quickly and takes a heavy toll of silkworm and even results in total crop failure when the infection is severe, causing huge economic loss. It is very unfortunate that, there is no precautionary measures to prevent the spread of microsporidians either vertically or horizontally, therefore the development of resistant genotypes is the only effective strategy to effectively control the economic losses.

Among numerous silkworm *B. mori* breeds, most are susceptible to microsporidian infections. These microsporidians are spore-forming unicellular parasites that have a unique ultrastructure and life cycle and infects host cells through the polar tube which is ejected when microsporidia are stimulated by suitable conditions, and the infective sporoplasm is transferred into the host cells through the polar tube prior to proliferation. The CSR2 is a commercial productive silkworm breed that is highly susceptible to microsporidian infections. However, the silkworm which exhibited the most resistance/tolerance to microsporidians is “Lamerin” or “Leimaren”, which belongs to Manipur, North East India is a commercially exploited breed. It is also the only breed of the silkworm *B. mori* which survives microsporidia infections. Though preliminary work carried out at SBRL showed 65% of the Lamerin breed were resistant and 35% were tolerant to microsporidia infection, the reasons for the said tolerance/resistance exhibited by Lamerin at the molecular level is not known. The proposed work thus aims to compare the gene expression profiles in two important silkworm breeds that is resistant and the other is susceptible to microsporidian infections. Hence, the present project deals with analyzing two contrasting bivoltine breeds of silkworm *B. mori* (Lamerin and CSR2) to identify and evaluate the resistance/tolerant genes/miRNAs with potential functions against microsporidian infections

Materials and Methods

Collection and rearing of silkworms for bioassay and tissue collection

The Lamerin, is a hibernated multivoltine, breed of silkworm was procured from our Research Extension Centre, Central Silk Board, Mantripukhri, Imphal, Manipur. The lamerin breed and the CSR2 breed (commercial bivoltine silkworm breed) was maintained at our laboratory. The larvae of both breeds were reared on mulberry leaves under stable 14 h light and 10 h dark at $25 \pm 1^\circ\text{C}$ and $75\% \pm 3\%$ relative humidity.

Bioassay studies and tissue collection for differential gene expression analysis

The freshly moulted healthy second instar silkworm larvae were collected from both breeds and were starved for six hours and divided into control and infected groups in triplicates. The larvae from the infected groups were fed per os via ingestion of a 3-cm² piece of mulberry leaf coated with 1×10^5 spores/ml of a highly virulent microsporidian, *N. bombycis* spores. The control groups were treated with sterile water. After the inoculated larvae consumed the entire leaves smeared with the spores, both the groups were reared on fresh mulberry leaves as per the feeding, cleaning, and sanitation schedules until the onset of spinning of cocoons (approximately 12-14 days). Further, approximately 10 larvae were collected at different time points starting from 12, 24, 48, 72, and 96 hr post-infection, from control and infected batches to analyze the copy number of the β -tubulin in both the breeds as well as for gene expression studies. The remaining larvae were continued for rearing till spinning stage to check the survivability. The mortality and the visual symptoms were recorded in control and infected batches.

For molecular analysis, the midgut tissue from larvae (10 nos.) from each time point was dissected out, for genomic DNA and total RNA isolation. For total RNA isolation the dissected tissue was washed in

DEPC treated water, suspend in Trizol reagent (Invitrogen) and kept at -80°C. The genomic DNA and the total RNA was extracted using Qiagen DNA & RNA extraction kits, according to the manufacturer's protocol. The purity of all RNA samples was assessed at an absorbance ratio of A260/280 and A260/230, and the integrity of the RNA also be confirmed by 1% agarose gel electrophoresis. Each RNA sample (1 µg) was used for cDNA synthesis in a 20-µL reaction mixture as per manufacturers instruction kit (prime script cDNA synthesis kit) (Takara) and 1-2ul of cDNA was used for semi quantitative analysis.

Collection of samples for Transcriptome and miRNA analysis

The fifth instar larvae (Day 1) of CSR2 and Lamerin breeds will be starved for six hours and then fed orally with 5000 spores/larva of highly virulent microsporidian, *Nosema bombycis* spores smeared on mulberry leaves. The control groups will be treated with sterile water. Subsequently the larvae will be reared on normal mulberry leaves till spinning. After 6hr post infection, larval midguts (10 Nos) will be dissected out, washed in DEPC treated water, mix and suspend in Trizol reagent (Invitrogen) and keep at -80°C. The dissected mid-guts from both the control and infected samples from CSR2 and Lamerin breeds will be subjected to RNA/miRNA sequencing, sequence analysis, assembly and annotation (Illumina Sequencing, read assembly and annotation) will be outsourced. Annotated 3'UTR of silkworm protein coding genes will be used for miRNA target prediction and function using silkworm genome database/miRBase/and standard softwares of the outsourced vendor.

Standard curve generation for qPCR analysis of β-tubulin' copy number in the infected sample

In order to determine the multiplication of *N. bombycis* in the infected samples of CSR2 and Lamerin, it is essential to generate the standard curve using the β-tubulin, gene which is the conserved across the *Nosema* sps. The *N. bombycis* genomic DNA was isolated from the infected samples and the genomic DNA was amplified using β-tubulin, gene specific primer (**Table 4**). The amplified PCR product was purified and cloned into pJET 1.2 vector and sequenced to confirm the cloned gene. The vector containing the cloned gene ie., the plasmid was inoculated to prepare the plasmid DNA for further use. The quantity of the plasmid DNA was quantified using nanodrop and copy number was determined using the following formula.

$$\text{Amount (ng)} \times 6.022 \times 10^{23}$$

$$\text{Length (bp)} \times 1 \times 10^9 \times 660$$

The number of β-tubulin gene copies in the sample was calculated using the formula and the plasmid was serially diluted to get different concentration of plasmid from 10⁷ copies to 10¹ copies was prepared to generate a standard curve. To check the copy number in the infected samples, the genomic DNA was isolated from the infected midgut tissue of both CSR2 and the Lamerin using 2PK buffer containing 200mM Tris, 25mM EDTA, and 300mM NaCl and 2% SDS followed by proteinase K for 30mins and DNA was extracted with phenol:chloroform: isoamyl alcohol (25:24:1), precipitated in 2.5 volumes of ethanol,

washed with 70% ethanol, and resuspended in 10mM Tris–1mM EDTA. The isolated genomic DNA was used for qPCR analysis of β -tubulin' copy number at different time points in the infected samples.

Differential gene expression of *N. bombycis* and host immune response genes in pebrine infected and control silkworms.

To analyze the differential gene expression of both *N. bombycis* specific genes as well as host immune response genes, total RNA was extracted from the infected as well as control midgut samples of *B. mori* by using Trizol reagent (Takara) according to the manufacturer's instructions. The quantitative PCR was done using gene-specific primers with 2 μ L of cDNA as a template in 20- μ L reaction mixtures using SYBR Premix Ex-TaqII (Takara), to determine the differential expression of *N. bombycis* specific and host immune response genes in the control and treatment groups. For qPCR, studies included initial denaturation of 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C in triplicate. The relative fold change in gene expression was calculated; the housekeeping gene GAPDH was used as an internal control to normalize the reactions. The gene specific primers used in the study is given in the table (Table 4).

Table 4: List of oligonucleotide primers designed for the screening the essential and early genes of *N. bombycis* infection in silkworms and silkworm host immune response genes (Nb: *Nossema bombycis*, Bm: *Bombyx mori*)

Target	Forward primer (5'-3')	Reverse primer (5'-3')
1 β -tubulin' (Nb)	CTTTGGACAATCTGGTGCTG	GAGAAGGGTTCCCATTCCTG
2 PTP1 (Nb)	CGGTGGTTTCCCAAGTAATG	AGGATGAGAGCCTGATGGTG
3 PTP2 (Nb)	TTCCTAACCGTGCAACAA	TGCTGTTTCAATGGCTTGAG
4 SWP 5 (Nb)	AATGCCGAAGCTCAAAAAGA	GCAAACACAGCAAGAATGGA
5 Gloverin -4 (Bm)	TGGGCCAATAAGAATGCGGA	TGTGCTTGATGGGATCTACTGG
6 Cecropin D (Bm)	AATACGACGCAAGGAGGAGG	ACACGCTAGGATGAAGCTGA
7 Serine protease 1 (Bm)	GCGGCCGAGGTACCAGGATT	CGGAGCGGGTGTTGGTCAGT
8 Serine protease 5 (Bm)	TGGTGCTACCCCCATCTTGC	CGGCCGAGGTACCAAAAGCGA
9 Serine protease 11 (Bm)	TCGCGACCACCGACGTCCAA	TGGGGTGCTACATCGCTCGGA
10 Cuticular protein 1 (Bm)	GGTGGGTGTACCTGTTGAGG	GGTCCAAAGCCACCATCGTA
11 Cuticular protein 2 (Bm)	GGTGGGTGTACCTGTTGAGG	GGTCCAAAGCCACCATCGTA
12 Moricin (Bm)	AGGTCATAAGATGTCAACGTGAA	TGTCGTTGCAATCAATATATGTACC

Statistical analysis

Data are presented as mean $\pm$ standard error of three independent experiments with 50 larvae in each experiment. Statistical analysis was performed using ANOVA (<http://www.graphpad.com/quickcalcs/>) to determine whether significant differences existed between the control and infected samples as $P < 0.001$ and $P < 0.0001$ were considered as significant (represented by asterisks *** and ****, respectively).

Results

Collection and rearing of silkworms for bioassay and tissue collection

The Lamerin, breed of silkworm *B. mori* is a resistant/tolerant breed (Golden yellow cocoons) to microsporidia. The CSR2 breed, is most susceptible to microsporidia infection (white cocoons) but it is a commercial race with good silk quantity and quality. The Lamerin breed showed comparatively low pre cocoon and post cocoon characters (**Fig. 13a**) (**Table 5**), as far as the disease resistance is concerned Lamerin breed shows better tolerance/resistance to microsporidia while the latter is more susceptible to pathogens. The bioassay batches were observed after 10-12 days post infection, for visible symptoms & the survivability between both the breeds. The typical symptoms of pebrine infection like weak unequal larvae, failure to undergo moulting was observed in the early stages of infection, and in some larvae, there was formation of black spots on the cuticle at 5th instar. The results clearly indicated the infection of *N. bombycis* in both CSR2 and Lamerin breeds and the mortality observed in the CSR2 indicated severe infection. (**Fig. 13b**).

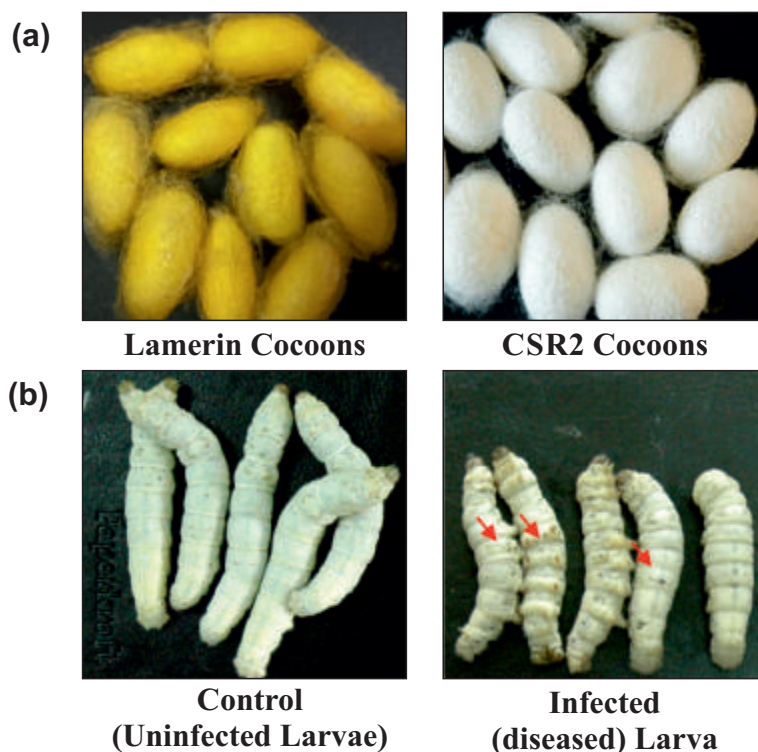


Figure 13: (a) Photographs of the Lamerin breed and CSR2 breed cocoons reared at Seri Biotech research Laboratory (SBRL) Bangalore; (b) Photographs of control and infected larva the red arrow on the photograph of infected larvae are the typical black spots on the cuticle of silkworm larvae and infected larvae showing retarded growth

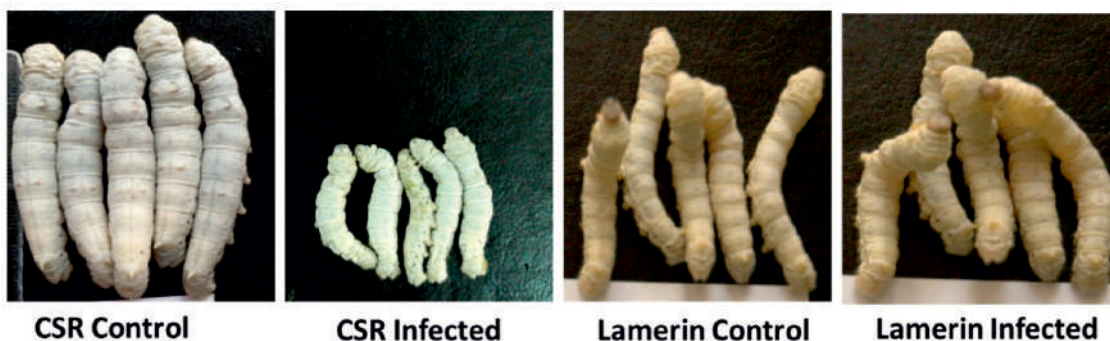
Table 5: Pre- cocoon characters of Lamerin and CSR2 breeds recorded during the silkworm rearing at SBRL

Race_name	Type of race	Country	Egg shape	Larval body colour	Larval pattern	Cocoon colour	Cocoon shape
CSR-2	Evolved	India	Ellipsoidal	Bluish white	Plain	White	Oval
Lamerin	Evolved	India	Ellipsoidal	Creamish white	Plain	Golden yellow	Flimsy without definite shape
Race_name	Fecundity (no.)	Hatching %	Wt. Of 10 grown larvae (gm)	Pupation rate (%)	Single cocoon wt. (g)	Single shell wt. (g)	Silk ratio (%)
CSR-2	484.00	86.90	35.34	89.08	1.66	0.38	22.97
Lamerin	322.00	90.05	19.64	81.44	0.94	0.13	13.51

3.2 Survivability analysis of the pebrine infected silkworm breeds

The survival analysis was done at the end of each instar starting from 3rd instar based on the number of larvae that survived and finally after the fifth instar the number of larvae that spun healthy cocoons were considered. The infection was given to freshly molt out third instar larva, at the end of the third instar >90% of the infected larva in CSR2 and Lamerin entered into next instar while remaining 10% larvae were weak & inactive. At 4th instar (5 days post infection) mortality was observed in the infected batches. The survival rate decreased significantly to 72.6 % in CSR2 and 87.3% in Lamerin breed. During 5th instar day 1 to day 06 mortality rate significantly increased and more number of larvae were found weak and inactive. At the end of 5th instar, the larvae in the infected batches showed typical infection symptoms like decreased size, weakened larva, with small black spots all over its body indicating typical pebrine infection and failed to spin cocoons (**Fig. 14a & b**). While the control batches, showed 85-90% survival between 3-5th instar in CSR2 and Lamerin. The overall bioassay results indicated 85% survivability of the Lamerin breeds to pebrine infection, while in CSR2 the survivability was less than 5% showing the highly susceptible condition in the CSR2 breed.

a.



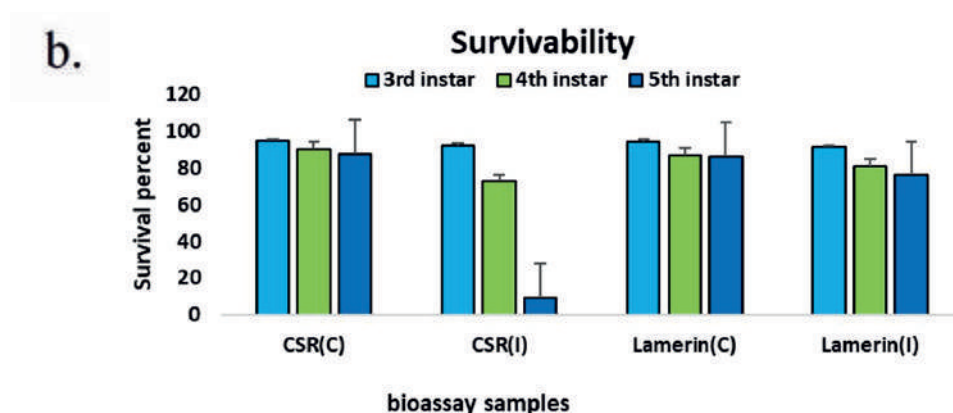


Figure 14: (a) Comparison of CSR2 and Lamerin uninfected and infected silkworms at the end of 5th Instar larva. The black spots are the typically symptoms of the pebrinedisease (red arrow) (b) Analysis of survivability of *N. bombycis* infected CSR2 and Lamerin Breeds at different instars.

Copy number of β -tubulin genes at different time points in the *N. bombycis* infected larvae

The standard curve developed for the analysis of copy number in the infected samples was utilized for the analysis of multiplication of *N. bombycis* in the CSR2 and the Lamerin breed at different time points. The standard curve generated was able to identify the copy number as low as 102 copies of beta tubulin in the infected samples. From the copy number studies, we observed >100 copies of β -tubulin in CSR 2 at 12hpi that increased to >8000 copies at 96hpi. However, in Lamerin we observed >15copies at 12hpi and at 96hpi the β -tubulin copy number was approximately 100 copies (**Fig. 15**). From the results it can be observed that initially the pathogen multiplication started as early as 6hpi, in both the breeds, gradually there was a significant difference in the rate of multiplication of *N. bombycis* in both the breeds. Further, at the 96hpi <100 copies of β -tubulin were observed in Lamerin when compared to > 8000 copies in the CSR2 breed. The results indicated successful infection in both the breeds and high susceptibility of CSR2 breed for *N. bombycis* infection. This preliminary result indicates the resistance shown by the Lamerin breed against the *Nosema bombycis* infection, may be due to the upregulation of immune genes at the early stage of infection thereby preventing multiplication of pathogen inside the host. While in CSR2, which is most susceptible to diseases showed signs of infection at a very early stage due to failure in the recognition of the pathogen at the early stage and suppression of immune responses of the host by the pathogen.

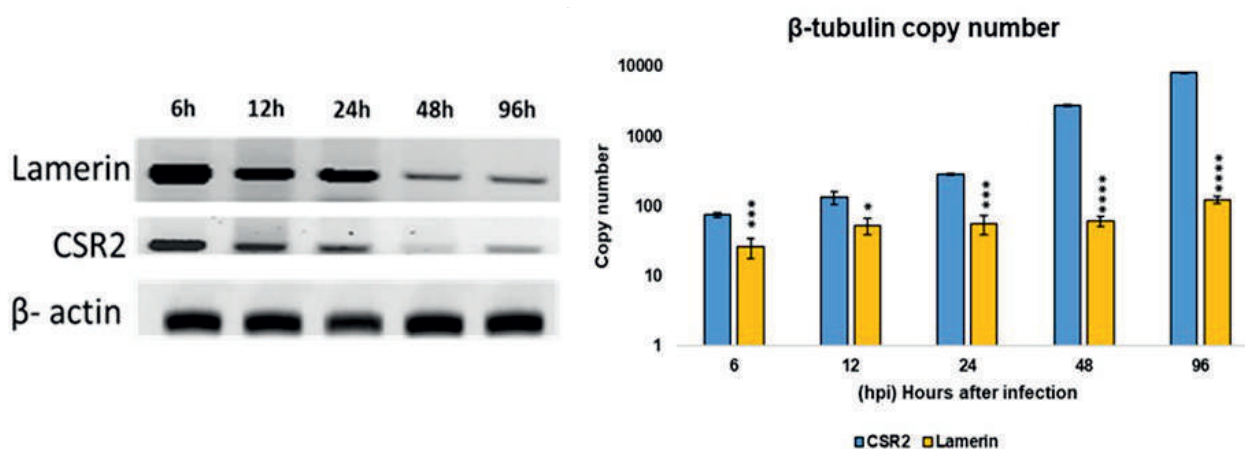


Figure 15: qPCR analysis to determine the copy number of β -tubulin in CSR2 and Lamerin at different time points.

Transcriptome analysis and validation of shortlisted immune genes using qRT PCR analysis

The freshly molt third instar larvae (Day 1) of both breeds was starved for six hours and then fed with 1×10^5 spores/ml of highly virulent microsporidian, *Nosema bombycis* spores smeared on mulberry leaves to imitate the natural infection process. The control groups were treated with sterile water. After infection, larvae are reared on normal mulberry leaves. One set of larvae were collected at different time points starting from 12, 24, 48, 72 and 96 hours post infection, to isolate genomic DNA and Total RNA for gene expression studies. The 24hr infected and the control samples from CSR2 and Lamerin in triplicates were sent for transcriptome and miRNA analysis through outsourcing. The larval midguts (10 Nos) were dissected out, washed in DEPC treated water, and suspend in Trizol reagent (Invitrogen) and keep at -80°C . Total RNA was extracted using Qiagen RNA extraction kits for both, according to the manufacturer's protocol. The purity of all RNA samples were assessed at an absorbance ratio of A260/280 and A260/230, and the integrity of the RNA also be confirmed by 1% agarose gel electrophoresis. The cDNA was synthesized using the standard protocol. From the transcriptome data and the *in silico* differential gene expression analysis 14 immune genes that are differentially expressed in the *in silico* analysis were shortlisted for validation in CSR2 and Lamerin breeds at different time points. In order to validate the results from the mRNA the relative expression levels of the selected genes that might be involved in microsporidia resistance was done by reverse transcription quantitative PCR (RT-qPCR) with SYBR green. The total RNAs from midgut of both strains was used for cDNA synthesis and PCR. The relative quantification using comparative $\Delta\Delta\text{Ct}$ method was done. The expression levels will be normalized using actin B gene. The experiments were performed in triplicate. The genes that are shortlisted for validation is indicated in the **Table 6 (Fig. 16)**.

Comparison Upregulated Downregulated

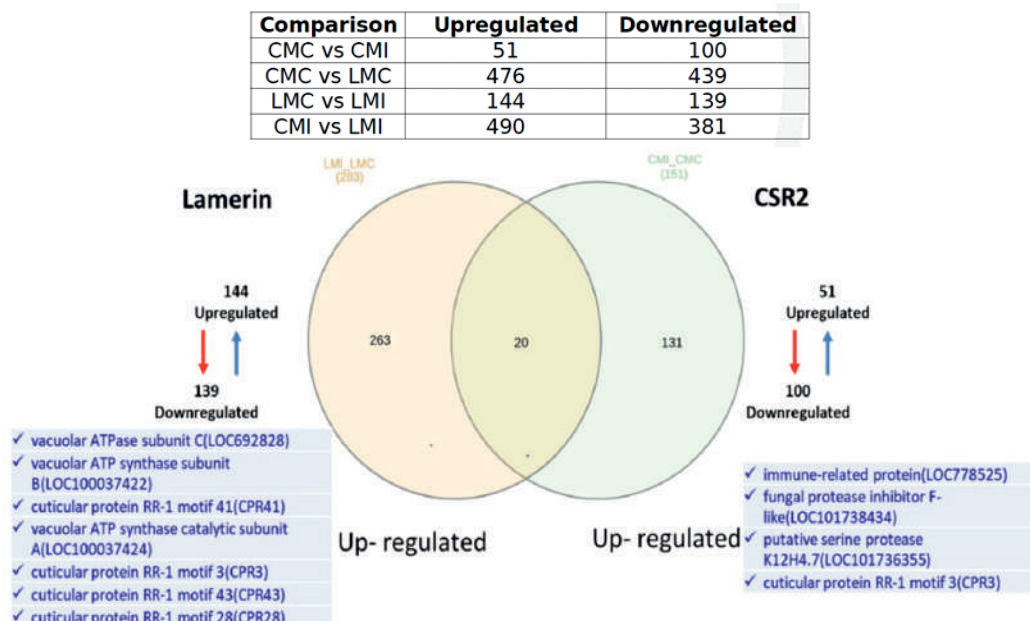


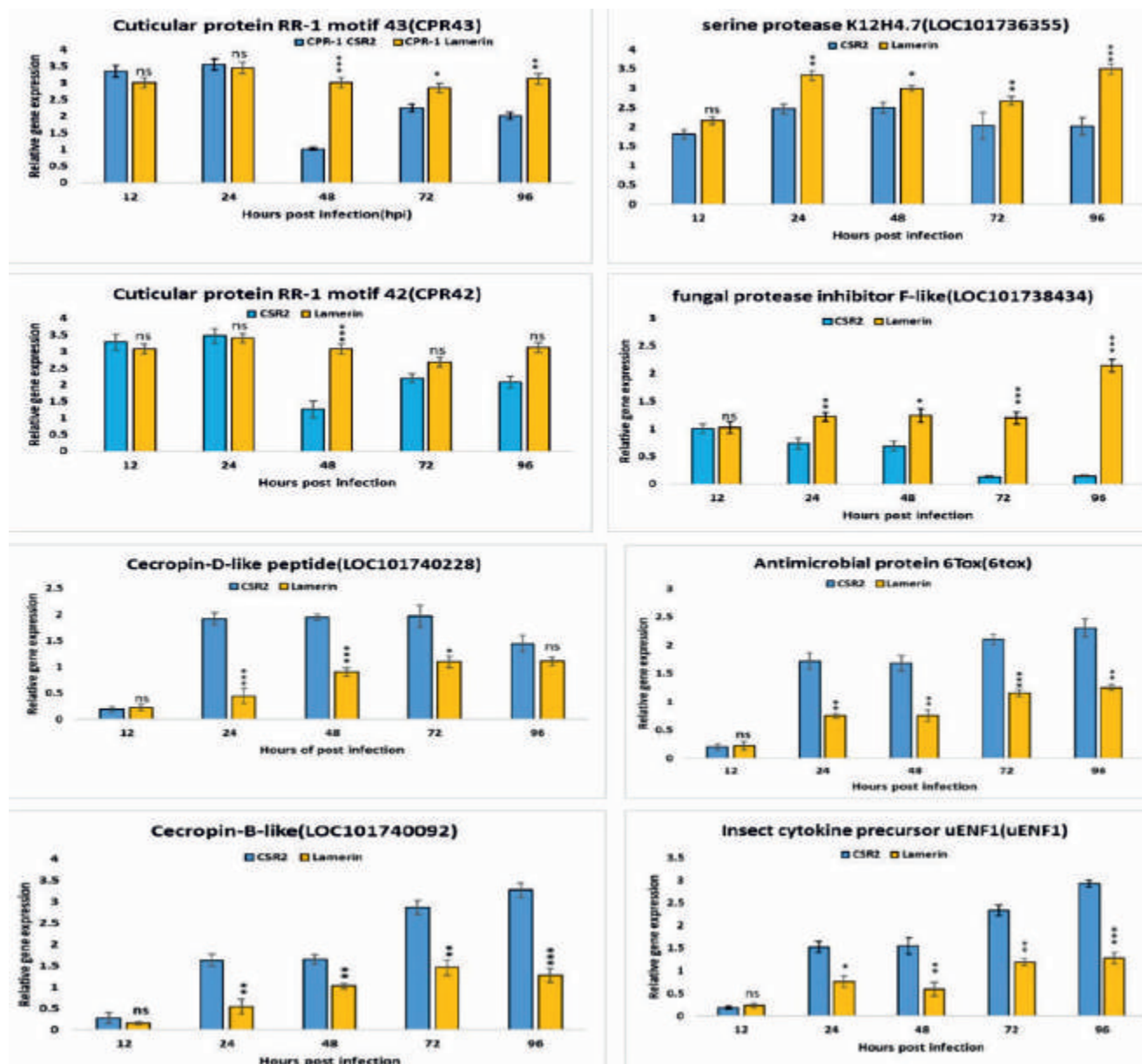
Figure 16: General overview of the differentially expressed genes in the control and infected samples of CSR2 and Lamerin

Table 6: List of immune genes selected for the validation in CSR2 and Lamerin at different time points.

Immune-related-Genes	Associated-miRNA	LMI-LMC	CMI-CMC	LMI-CMI	LMC-CMC	Gene names
NM_001043393.1	bmo-mir-3207			upregulated	upregulated	peptidoglycan recognition protein S6(PGRP-S5)
NM_001043419.1	bmo-mir-2742	upregulated				alpha-tubulin(Tua1)
NM_001043997.1	bmo-mir-989a	downregulated	downregulated	upregulated	upregulated	cecropin A(CecA)
NM_001098358.1	bmo-mir-3301	upregulated				vacuolar ATP synthase subunit B(LOC100037422)
NM_001098359.1	bmo-mir-2782	upregulated				vacuolar ATP synthase catalytic subunit A(LOC100037424)
NM_001168283.1	bmo-mir-3253	downregulated				integrin beta subunit 2(LOC100313953)
XM_004931071.4	bmo-mir-3327-5p	upregulated				V-type proton ATPase 116 kDa subunit a1(LOC101742516)
XM_012689821.3	bmo-mir-2778a-2-5p			downregulated	downregulated	nitric oxide synthase 2(NOS2)
XM_012690766.3	bmo-mir-3221			downregulated	downregulated	nitric oxide synthase 1(NOS1)
XM_012692582.3	bmo-mir-932	upregulated				tubulin beta chain(LOC101744447)
XM_012694266.3	bmo-mir-3383-5p			upregulated		fibroinase(Bcp)
XM_012694918.3	bmo-mir-3385-5p			upregulated	upregulated	beta-1,3-glucan recognition protein 3(BGRP3)
XM_012696685.3	bmo-mir-71-5p			upregulated		homer protein homolog 2(LOC101740566)
XM_038010817.1	bmo-mir-3427			downregulated		insulin receptor(InR)
XM_038016618.1	bmo-mir-2733c	upregulated				actin A3(A3)
XM_038019252.1	bmo-mir-2798	downregulated		downregulated	downregulated	scavenger receptor class B member 1(LOC101740110)

Validation of Differential gene expression of host immune specific genes in CSR2 and Lamerin

In this present experiment, we have analysed the differential gene expression both semi-quantitatively and quantitatively of host immune specific genes. qPCR was done to investigate gene expression patterns in the midgut of CSR 2 (susceptible) and Lamerin (tolerant/resistant) infected with



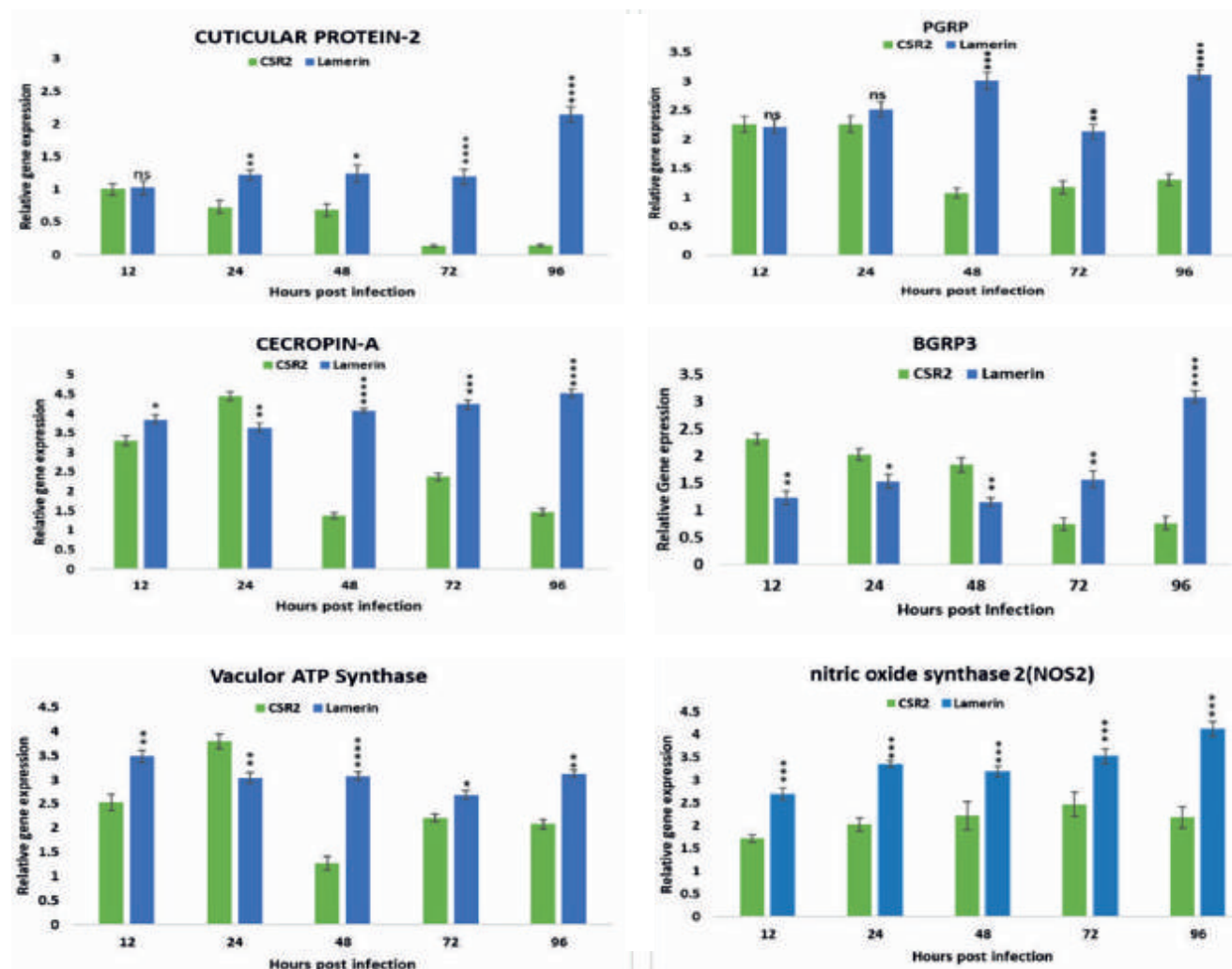


Figure 17: Quantitative PCR analysis *N. bombycis* infected midgut tissue RNA of CSR2 (A) and Lamerin (B) with different *host* immune response genes. The RNA of orally infected 3rd instar larvae was collected at different time points starting at 12, 24, 48, 72, 96, hours post infection (hpi)

the microsporidian *N. bombycis*. From the qPCR analysis it was observed that the expression of cuticular protein genes was same in both the breeds at 6hpi however after 24hpi till 96hpi the expression of cuticular genes increased significantly ($p > 0.001$) in the Lamerin breed, but in CSR2 the expression of cuticular genes decreased across different time points. The peptidoglycan recognition protein (PGRP) expression at 6hpi -12hpi was same in both the breeds however at 72hpi the expression of PGRP decreased and increased significantly at 96hpi in Lamerin breed, but in CSR2 the expression of PGRP decreased from 48hpi to till 96hpi. The expression of Cecropin A increased gradually from 6hpi to 12hpi in CSR2 but further there was no increase the expression of the gene, in contrast in Lamerin breed

initially at 6hpi-12hpi there was only slight increase in expression, but gradually the expression increased from 48hpi to 96hpi. The B 1-3 glucan recognition protein expression was comparatively higher in the CSR2 from 6hpi till 48hpi, while the expression of BGRP was lower in the Lamerin breed during the same hpi however the expression was significantly increased from 72hpi till 96hpi in the Lamerin, but there was no increase in the expression in the CSR2. The expression of NOS2, Nitric oxide is a reactive free radical mediating in neurotransmission, antimicrobial and antitumoral activities, the expression of this gene in CSR2 was significantly lower from 6hpi-96hpi, in contrast the expression this gene increased gradually from 6hpi till 96hpi in Lamerin (**Fig. 17**). Therefore, the overall differential gene expression of immune genes in both the breeds at different time points indicates that, there is definitely an initial response from the host against the invading pathogen which is evident from the increased expression of most of the genes at 6hpi except for NOS genes in CSR2.

Output/Conclusion

From the earlier bioassay studies, it was observed that the survivability rate of Lamerin breeds was > 70% in comparison with < 10% survival rate in the CSR2, however, the molecular evidences for the difference in the survivability were lacking. Therefore, in the present study, the bioassay results were supported by the transcriptome data and quantitative PCR. The results showed significant higher expression of host specific immune response genes. It was also observed that in CSR2 at 48hpi most of immune response genes were downregulated and again decreased at 96hpi and there was also increase in the expression intermily this indicates the activation of Toll and IMD pathway of the host immune system to activate immune response. The results were evident to show that the Lamerin breed was able to survive with the infection like other wild species of silkworm that exists with the pebrine infection due to its adaptability to the infection through strong immune genes. The study showed the upregulation of specific antimicrobial genes, serpins in response to microsporidian infection. The upregulation of these genes in Lamerin breed in comparison to CSR 2 is an indicative of the tolerance/resistance exhibited by the Lamerin breed against the *N. bombycis* infection. The upregulation of immune genes in the Lamerin breeds may be attributed to the genetic make-up of the breed and its adaptability. This can be attributed to the fact that the Lamerin breed is generally wild species found in the Manipur region, as they are wild, they have lot of challenges posed by the biotic factors most importantly attack from pathogen, therefore in order to combat the infection and survivability in the wild the genetic make-up of the Lamerin breed has evolved to survive the infection.

The future line of work will be focused on miRNA analysis among both the breeds post infection with *Nosema bombycis* to study the changes in the expression levels of messenger RNAs (mRNAs) and non-coding RNAs, including microRNAs (miRNAs) following infection will provide vital information miRNA associated with the immune response as well as on host defense strategies against invading pathogens.

2. AIT08006EF: Development of lateral flow assay kit for detection of pebrine disease in silkworm

(Funded by DBT-BIRAC, New Delhi in collaboration with Bhat Biotech Ltd, Bangalore;

Duration: March 2021- January 2023)

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Shama Bhat³

¹CSB-CTRTI, Ranchi; ²CSB-SSTL, Kodathi; ³Bhat Biotech Pvt. Ltd., Bangalore

Objectives:

1. To characterize essential genes of microsporidians infecting *Antheraea mylitta* and *Antheraea assamensis* for identifying candidate target genes for early diagnosis.
2. To develop Lateral Flow Assay LFA and to optimize the kit for detection of microsporidiosis in silkworm *Bombyx mori* as well as vanya silkworms.
3. To validate the optimized LFA kit to detect microsporidian infection in silkworm *Bombyx mori* as well as vanya silkworms at field level.

Pebrine is a devastating disease which affects silkworms. The disease is caused by notorious genus under microsporidia called *Nosema*. The disease displays horizontal as well as vertical transmission from mother moth to offspring. Mother moth examination under the microscope is the only method followed across seed centers, which is cumbersome, highly error prone and often does not pick in case of mild infection. There are many PCR based techniques have been developed, however these methods require expertise and is limited at laboratory level. In this study, a novel Lateral flow assay LFA has been attempted to detect the pebrine disease at field level. Lateral flow assay is a novel chromatographic immunoassay, a device based on the principles of a simple biosensor with low cost of production, ease of application and simplicity and therefore suitable for high throughput screening. Spore wall proteins (SWPs) are considered to be a vital component of the microsporidian spores. SWPs are found either on exospore or endospore and can have many important functions during infection to the host. In this project, we have identified, cloned and expressed the spore wall proteins of *Nosema assamensis*. These spore wall proteins were identified to be suitable molecular markers to detect microsporidian infection in silkworms. The recombinant proteins were expressed in *Escherichia coli* expression system using efficient expression method through IPTG induction (**Fig. 18A, B**). The molecular size of *SWP1*, *SWP3* and *SWP5* were found to be 46 kDa, 52kDa and 35 kDa respectively (**Fig. 19**). The purified spore wall proteins were produced in bulk. The oligomeric state of *SWP1* protein was determined using molecular weight of standards where, the peak of *SWP5* corresponded to ovalbumin peak at 46 kDa, suggesting the *SWP5* to be a monomer. Purified recombinant protein *SWP1* was further used in raising antibodies in mice. After administration of 3rd booster dose of *SWP1*, significant titer values were noticed (**Table 7**).

The titre values range from 0.7 to 1.7 at 1:16k concentration and 0.39 to 1.1 at 1:32k dilution these values were significantly higher than 1st and 2nd booster doses. The antibodies were also used in Immunofluorescence assay (IFA) where, *SWP* was found to be localized on exospore and polar tube hence, indicating its probable role in host pathogen interactions during spore germination. The *SWPs* from the infected silkworms were also successfully used in Immunoprecipitation assay (IPA) which proved antibody specificity towards the antibodies raised in rabbit. It is evidenced that *SWP1* is an ideal target for LFA assay since, it is localized in the exospore and has a role in the extrusion of the polar tube.

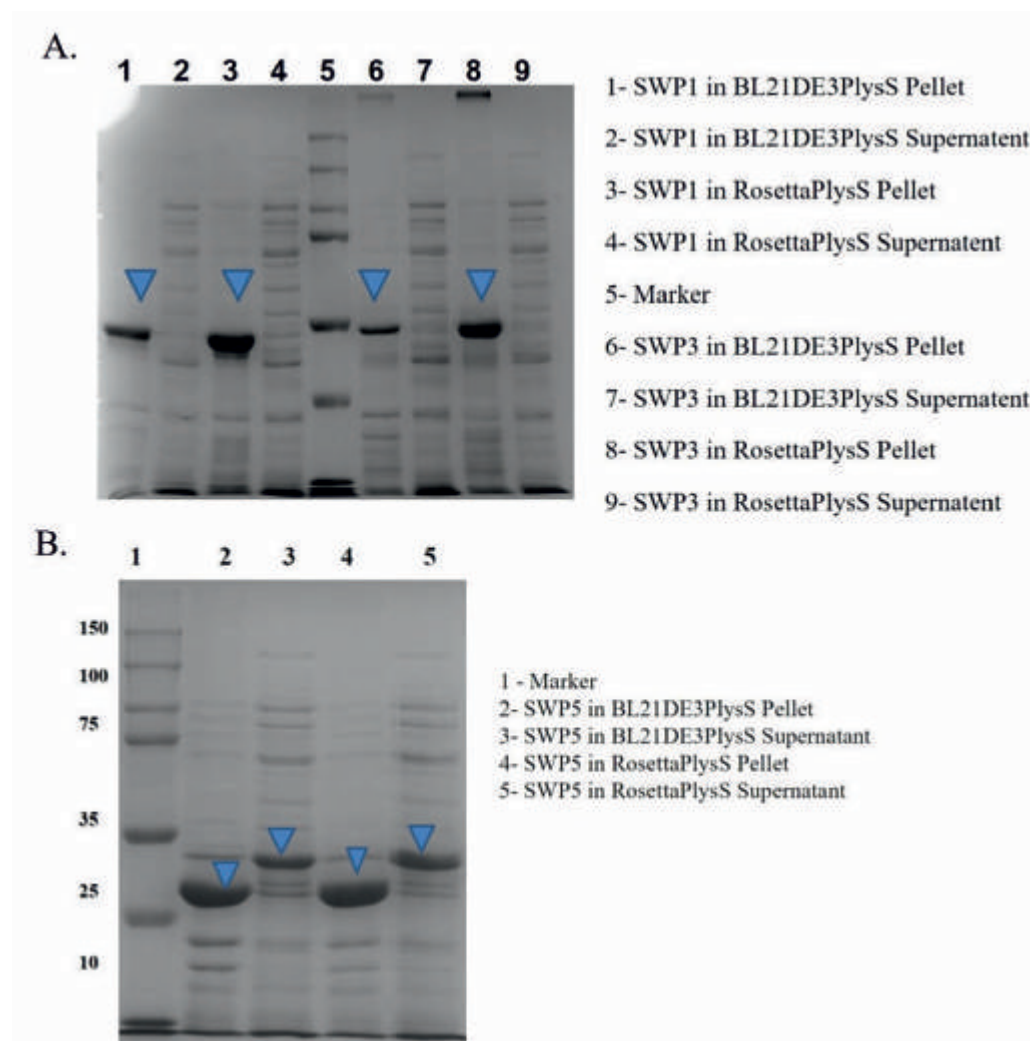


Figure 18: Expression of recombinant spore wall proteins of microsporidia. **A:** Spore wall protein 1 (SWP1) and 3 (SWP3); **B:** Spore wall protein 5 (SWP5)

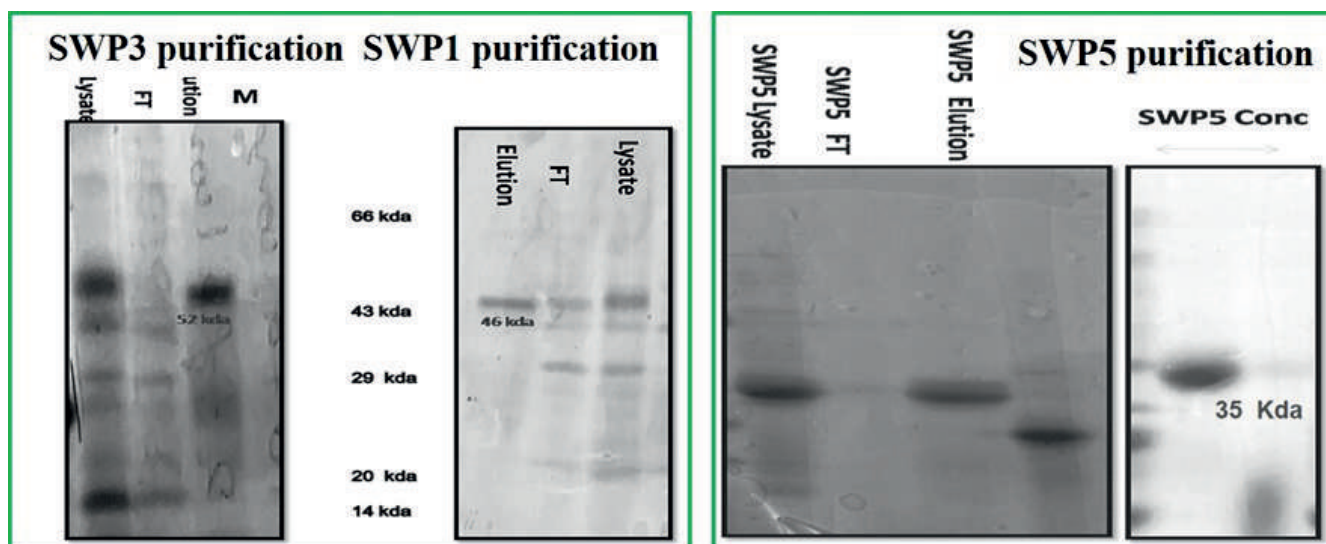


Figure 19: Purification of recombinant spore wall proteins, SWP1, SWP3 and SWP5.

Table 7: Antiserum ELISA titration for monoclonal antibody

Dilution	Mice-1	Mice-2	Mice-3
1:1k	2.243	2.64	2.67
1:2k	1.732	2.7	2.1455
1:4k	1.4455	2.735	1.6805
1:8k	0.985	2.3005	1.1625
1:16k	0.704	1.7375	0.803
1:32k	0.3985	1.1385	0.4835
1:64k	0.2385	0.791	0.263
1:128k	0.1205	0.368	0.1285
1:256k	0.0705	0.256	0.0855
1:512k	0.0435	0.165	0.054
1:1024k	0.027	0.097	0.0345
Negative	0.013	0.0125	0.11

Antibody generation

Polyclonal antibodies against *Nosema* SWP1 were generated in 12 weeks old rabbit. The antigen was provided to the rabbit intramuscularly for 45 days at an interval of 15 days. The sera collected. after 45 days was used in immunoassay studies. The SWP1 protein in Freund's complete adjuvant was injected for the first immunization. Then, SWP1 protein in Freund's incomplete adjuvant was used as immunogen for the second and third immunization with respective intervals. Sera taken on day 0 were used as pre-immunized antibody. Seven days after the third immunization, blood samples were taken to determine antibody titre by Enzyme linked immunosorbent assay (ELISA). Eight-week-old BALB/c mice were immunized with 100 ug of recombinant SWP 1 protein at 1:1 with FCA (Freund's Complete Adjuvant), and three more immunizations were performed every 2 weeks using a mix containing 100µg of SWP1 at 1:1 in FIA (Freund's Incomplete Adjuvant). Four days after the last immunization, the spleen was isolated and splenocytes were fused with goat myeloma cells using 50% polyethylene glycol; the cells were suspended in hypoxanthine-aminopterin-thymidine (HAT) medium and directly plated in 96-well plates. The plates were incubated in CO2 incubator at 37 °C for 5 to 6 days. At day 6/7, the HAT selection medium was removed and the cells were fed with fresh HT medium. Detailed immunization schedule is furnished. SWP1 (1ng/100 µL) was used for screening in ELISA experiment. Determination of antibody titre and sensitivity of the assay was determined by direct antigen coating in ELISA plates.

ELISA

Recombinant SWP1 as well as Pebrine infected silkworm samples were used as antigen in ELISA. The infected silkworm larvae samples and healthy silkworm larvae samples were crushed with micro pestle in the water. 100 µl of the crushed sample was loaded into the wells of ELISA microtiter assay plate. Further, 100 ng/well of recombinant SWP1 was coated. 50µl of healthy silkworm larvae/ uninfected silkworm larvae samples of unknown titre was used as a negative control. BSA devoid of tag was used as a negative control. The ELISA plate was incubated overnight at 4°C in the refrigerator. The non-specific sites in the plate were blocked using blocking solution 5% Skim Milk powder in PBS for 1 h at RT. The wells were washed with 250 µl PBST buffer thrice after each step. 100 µl of primary antibody (anti-SWP1 antibodies in ratio 1:1000) was loaded in each well. 100µl of secondary antibody (goat anti-rabbit immunoglobulin IgG conjugated to HRP in ratio 1:5000) was loaded in each well of the ELISA plate. The plates were incubated for 1 h at RT each. A colour reaction developed with addition of 100 µl of tetramethylbenzidine (TMB) in each well was read after the reaction was stopped by addition of acid at 450 nm in a Microplate ELISA Reader.

The Dot blot immunoassay

Dot-blot assay was carried out using HRP conjugate antibodies. For this, SWP1 (100 µg/ml) was spotted onto the piece of nitrocellulose membrane. The membrane was air dried and subjected to blocking solution for 2 h at RT. The membrane was incubated with primary and secondary antibodies for

1 h at RT each. The membrane was washed thrice with 1X PBST after each step. The dot blot was developed by using 3, 3'- Diaminobenzidine (DAB), developing solution. BSA without his tag and devoid of any tag were used as Negative control.

Development of Lateral flow assay (LFA) kit

LFA kits were prepared in Bhat Bio-Tech India (P) Ltd for the detection of *Nosema* infection in silkworms. *Nosema* sp. infected samples were tested with the LFA kit as per the manufacturer's protocol.

Validation of LFA kits

The developed LFA kits were validated with *Bombyx mori* silkworms. The experiments shown positive results with *Nosema bombycis* infected silkworms samples in which colour development was seen in the form of two bands on LFA pad. Further, the developed technology was demonstrated to seri-stake holders such as silkworm rearers and Chawlki centers

Results

Purified recombinant protein SWP1 was used in raising polyclonal and monoclonal antibodies. These antibodies were able to detect homogenized SWP1 even at low concentration of 100 pg/ml in ELISA. Evaluation of antibodies produced against SWP1 by ELISA indicated that the 2nd and 3rd bleeds contain highest titers of SWP1 antibodies. After administration of 3rd booster dose of SWP1, a significant ELISA titer values were noticed (**Table 8**) with both polyclonal and monoclonal antibodies (**Table 9** and **10**).

The recombinant SWP1 shows high binding for polyclonal anti-rabbit antibodies even at low concentration. Even at 1:1,28,000 dilution, significant titre is observed (0.6, where as SWP1 from infected sample binds at 1:16000 dilution ratio. ELISA experiments are positive for detection of SWP1 in infected silkworm samples. The results indicate an apparent titer with 1:8000 dilution with polyclonal antibodies. Dot-blot technology markedly saves time, since it does not require chromatography, gel electrophoresis or complex gel blocking procedures. The experiment was performed with using anti-rabbit antibodies and anti-HRP conjugates. Our experimental results provided evidence for the specific binding of both polyclonal and monoclonal antibodies for recombinant SWP1 in Dot-blot assay. Dot blot experiment further confirms the presence of SWP1 in recombinant as well as infected sample

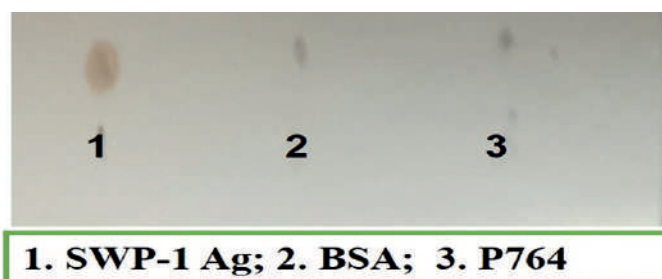


Figure 20: Dot blot assay of SWP1 using monoclonal antibodies using polyclonal and monoclonal antibodies (**Fig. 20**). BSA and in-house P764 protein serves as negative control in the experiment. Experiments with LFA kit shown positive results with *Nosema bombycis* infected silkworm samples in which colour development was seen in the form of two bands on LFA pad (**Fig. 21**)

Table 8: Antiserum ELISA titration for polyclonal antibody- SWP1

Dilution	Titre value
1:1k	2.6805
1:2k	2.6765
1:4k	2.6365
1:8k	2.6165
1:16k	2.6105
1:32k	1.842
1:64k	1.13
1:128k	0.6015
1:256k	0.3305
1:512k	0.185
1:1024k	0.101
Negative	0.013

Table 9: Antiserum ELISA titration with monoclonal antibody- SWP1

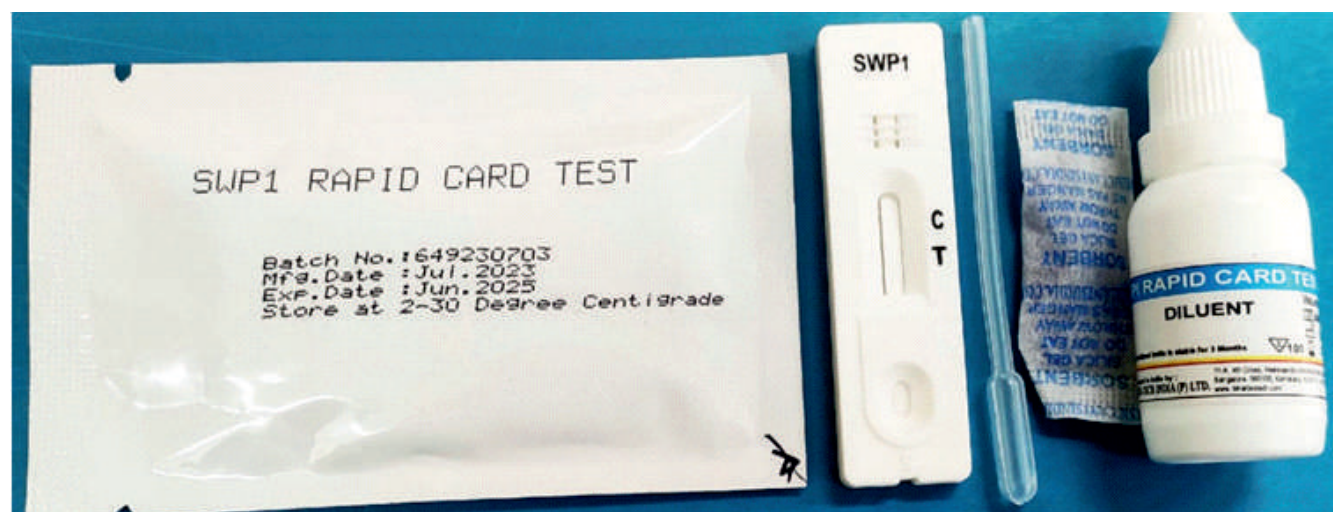
Dilution	Titre value
1:1k	2.6335
1:2k	2.607
1:4k	2.544
1:8k	1.989
1:16k	1.2755
1:32k	0.8195
1:64k	0.433
1:128k	0.235
1:256k	0.136
1:512k	0.076
1:1024k	0.0445
Negative	0.017

Table 10: ELISA assay for diagnosis of SWP1 in infected silkworm samples

Dilution	Titre value with infected samples
1.1k	1.014
1.2k	0.5435
1.4k	0.284
1.8k	0.1315
1:16k	0.092
1:32k	0.045
1:64k	0.0305
1:128k	0.0225
1:256k	0.0205
1:512k	0.02
1:1024k	0.018
negative	0.016

The developed LFA kits were validated with Vanya silkworms (*A. mylitta*) silkworms. The experiments shown positive results with *Nosema mylitta* infected silkworms samples in which colour development was seen in the form of two bands on LFA pad (**Fig. 22**). The evaluation of developed technology for the detection of *Nosema* sp. infecting wild silkworms such as *Antheraea assamensis* (Muga silkworm) and *Antheraea mylitta* (Tasar silkworms) were included in OFT trials. The overview of the technology is shown in **Fig. 22**. Further, the developed technology was demonstrated in CSB units and to seriestakeholders such as silkworm rearers and chawki centers. The detection efficiency of the kit is found to be 88% when compared to conventional PCR targeting *Nosema* detection, however the color intensity of the bands needs to be optimized.

**Figure 21:** LFA kit developed for the detection of *Nosema* infection in silkworms. Colour development was seen both in negative samples (single band) and *Nosema* infected positive samples (two bands).



LFA sachet

LFA strip Dropper

Assay Buffer

Figure 22. Overview of the developed LFA kit for detection of pebrine

Outcome and utility of the project: The developed technology will be disseminated amongst the farmers and stakeholders involved in the mulberry and vanya sectors to increase silk production and, in turn, improve the socio-economic status of seri-farmers. The study has high potential in terms of commercialization since, sericulture being the primary employment for ~10 million farmers in India, the developed kit will not only be utilized by farmers but also suitable for seed production centers and CRCs. There is an ample opportunity to develop related diagnostic kits associated with other diseases in future where this study will provide the basic framework for generating efficient diagnostic kits.

TRANSFER OF TECHNOLOGY

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

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

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

*CSB-CSRTI, Mysore

Objective:

1. To evaluate the FC1RxFC2R 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 (Li et al, 2010)(Savanurmah et al, 1994). The survey of Flacherie disease on silk worm was carried out, during the year 2010-11 through 5 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

During the Phase I of the project BmBDV resistant homozygous lines of CSR6R and CSR26R were developed followed by the Phase II of the project AIT 08005MI: Development and Evaluation of Bidensovirus resistant silkworm hybrids developed from marker assisted breeding lines – Phase II , we have developed BmBDV resistant homozygous lines of CSR2R and CSR 27R that are resistant to BmBDV. All the resistant breeds/lines were developed through molecular marker assisted selection. Further, the parental breeds were crossed to develop FC1R and FC2R to produce double hybrids that were completely resistant to BmBDV infection. All the BmBDV resistant parents and the hybrids were tested at every stage for the presence of *nsd-2* marker and also through bioassay studies. The said breeds were also tested for the presence of marker and bioassay studies to validate BmBDV resistance in the developed breeds at CSRTI, Mysore before the preparation of FCs for the OST at different RSRS under CSRTI, Mysore.

Preliminary work done Phase II:

The data of the performance of the resistant double hybrids during 1st OST at RSRS stations showed >3% increase in the survivability in RSRS Chamarajanagar and Kodathi and the total yield/100DFLs was 69kg/100DFLs and 72.4kg/100DFLs respectively, while in RSRS Anantapur the BmBDV resistant lines showed 17% increase in the survivability compared to control batches and total yield was 78kg/100DFLs, in RSRS Salem though there was not significant difference in the survivability between the control and the resistant batches but the yield was 78kg/100DFLs in the resistant batches. The results obtained from the RSRS stations indicated significant improvement in the yield in the resistant lines compared to control during the rainy season between July to October when the viral flacherie incidences are predominant.

Sl. No.	Name of the technology	Progress Achieved
1	OFT for the evaluation of BmBDV resistant mulberry silkworm Double Hybrids at farmer's field.	a) Rearing of parental breeds, Bioassay for BmBDV resistance. b) Crossing of BmBDVresistant parents to develop foundation crosses for producing target no of DFLs is achieved. (CSRTI, Mysore) c) 8000DFLs of BmBDV resistant mulberry silkworm Double Hybrids are produced for the trials. d) PCR analysis to confirm the presence of BmBDVresistnce marker in the developed crosses and hybrids is completed.

Anticipated outcome

As per the proposed work plan 20000DFLs of the resistant double hybrid will be tested at 50 locations to evaluate the survivability and yield of BmBDV resistant double hybrids at farmers level.

Component II: OST for evaluation of BmBDV resistant mulberry silkworm hybrids at CSB/Dos stations. (Duration: July 2023-June 2025)

Tulsi Naik K S, Ramesha A, Rahul K.¹, Mihir Rabha¹, B. Vijaya Naidu², Harish Babu³

¹CSB-CSRTI, Berhampore, ²REC Bagmara, Mothabari, ³RSRS Kalimpong

Objectives: To assess the field performance of SK6RxSK7R resistant to BmBDV (Viral Flacherie) at designated stations under CSB-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 CSB-CSR&TI, 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 climatic conditions prevailing in the tropical regions are most unpredictable and high temperature as well as high humidity are the predisposing factors for the silkworm diseases caused by viruses, bacteria, fungi and microsporidia in bivoltine breeds. Among the silkworm diseases, viral diseases contribute to 70 per cent of total crop loss every year. Viral diseases caused by Nucleopolyhedrovirus (NPV) and Densovirus (DNV) (DNV-1 and DNV-2) in mulberry silkworms account for the majority of cocoon crop loss (Gupta et al. 2019). Among them, Densovirus-2 now designated as *Bombyx mori* bidensovirus is the causative agent of the “flacherie” disease with destructive crop damage due to fluctuating temperature and humidity as well as inferior quality of mulberry leaves fed to the silkworm. Hence, the developed breeds SK6R and SK7R as well as their hybrid will improve productivity due to its disease resistant trait particularly against *BmBDV* resistance which can be successfully commercialized in northeastern part of India. In this context, the improved SK6R and SK7R breeds can be tested under on station trials at designated locations in West Bengal regions, particularly RSRS/ REC stations to evaluate the performance of the developed breeds in comparison with the control.

Table 11: Performance of BmBDV resistant single hybrids during the OST Trials

A.	Trial of Technologies (for validation) .At CSB Units						
Sl. No.	Name of the technology	No. or quantity per trial location (DFLs/cuttings/sampling/seedling/formulation/product)	Target for 2023-24		Achievement during the quarter		Remarks
			Physical (No.)	Name of the location (s)	Physical (No.)	Name of the location (s)	
1	OST for evaluation of BmBDV resistant mulberry silkworm hybrids at CSB / Dos stations	5 DFLs/trial	3	CSRTI, Berhampore REC Motibhari, RSRS Kalimpong	3	CSRTI, Berhampore, REC Motibhari, RSRS Kalimpong	The hybrids were tested in spring and autumn(02 seasons) in RSRS Kalimpong and 03 seasons at CSRTI Berhampore and REC Motibhari. There was 9%, 5% and 4% increase in survivability compared to control in RSRS Kalimpong, CSRTI Berhampore and REC Motibhari respectively.

Anticipated Outcome

The OST data obtained after the trials particularly, the survivability of the BmBDV resistant hybrid in addition to the overall performance of the hybrid will lead to utilization of this hybrid for improved production of hybrids for development of multiXBivoltine crosses or Bivoltine hybrids (Table 11).

TRAININGS

The Institute has organized a 21 days training program entitled “**Molecular Techniques Applied in the Field of Sericulture**” from 04.12.2023 to 24.12.2023 in which 14 participants including Students/ Research Scholars/Assistant Professor from different state (Assam, Jammu & Kashmir, Karnataka and Kerala) participated.



SILKWORM STOCK MAINTENANCE

1. Six lines of Transgenic CSR4 and CSR27 breeds with NPV tolerance generated through RNAi.
2. Transgenic lines of CSR2 breed for higher immunity carrying over expressing immune genes.
3. Three lines of MASN silkworm breeds for NPV tolerance developed through marker assisted selection.
4. Transgenic CSR2 breed expressing fusion protein.
5. Four lines of bivoltine breeds with BmBDV resistance.

MEETING / WORKSHOP / CONFERENCE / SYMPOSIUM / SEMINAR

RAC Meeting conducted

The 31st & 32nd meeting of the Research Advisory Committee were held on 18th April 2023 & 18th October 2023 respectively wherein review of the outcome of concluded projects and evaluation of new project proposals and progress of ongoing projects was conducted under the Chairmanship of Dr. N. K. Krishnakumar, Former DDG (Horticulture), ICAR, New Delhi.

राष्ट्रीय राजभाषा तकनीकी सेमिनार

केंद्रीय रेशम बोर्ड के प्लैटिनम जुबली के अवसर पर, संस्थान ने "केन्द्रीय रेशम बोर्ड के 75 साल: उपलब्धियां एवं भावी परिदृश्य" विषय पर एक राष्ट्रीय राजभाषा तकनीकी सेमिनार का आयोजन 15 फरवरी 2024 को किया। इस कार्यक्रम में केंद्रीय रेशम बोर्ड के वैज्ञानिकों और कर्मचारियों द्वारा 61 शोध पत्र प्रस्तुत किए गए।



Meeting / Workshop / Conference / Symposium / Seminar Attended

1. Dr. G. Subrahmanyam and Dr. Himanshu Dubey attended online workshop on Genome informatics during 17th June to 15th July 2023, organized by EMBL-EBI, UK.
2. Dr. Himanshu Dubey attended online Faculty Development Program on “Frontiers in Life Sciences and value-added products from Bioresources” organized by Vignan's Foundation for Science, Technology and Research Guntur Andhra Pradesh and ICAR-IARI New Delhi from 12th to 17th February 2024.
3. All the Scientists of this Institute participated in International Conference on “Silkworm Seed Industry: Opportunities and Future Prospects, SILKWORM SEEDCON-2024” organized by National Silkworm Seed Organization (CSB-NSSO) on 30th & 31st January 2024” at National Institute of Fashion technology (NIFT), Bangalore, India
4. All the Scientists of this Institute organized and participated in राष्ट्रीय राजभाषा तकनीकी सेमिनार on 15 February 2024.

Abstracts published in Workshops/ Conference / Symposium / Seminar

1. Subrahmanyam G, Deepika I, Ponnuvel KM, Indira Kumar I, Singh A, Dubey H, Debnath R, Sivaparsad V, Moorthy SM (2024) Molecular and biotechnological interventions for management of cytoplasmic polyhedrosis in muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae) seed crop rearing in Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore
2. Debnath R, Kamatchi I, Sasaki K, Dubey H, Subrahmanyam G, Ponnuvel KM, Kobayashi J, Moorthy SM (2024) Construction and pangenome analysis reveal evolutionary dynamics in group-I Nucleopolyhedroviruses infecting Saturniid silkmoths in Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore
3. Himanshu Dubey, Indumathi Kamatchi B, Rajal Debnath, A Ramesha, G Subrahmanyam, S. Manthira Moorthy, KM Ponnuvel, and Kallare P Arunkumar (2024) Genomic Characterization of Vitellogenin and Vitellogenin Receptor Genes in *Antheraea assamensis* Helfer in Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore
4. Ramesha A, Naleen, Himanshu Dubey, Prashanth Sangannavar, Shreya Anthony, Raju Mondal, K.M. Ponnuvel, B.T. Srinivasa, V. Sivaprasad, R.K. Mishra, Tanmoy Sarkar (2024) Doubled haploids: Opportunities and challenges in mulberry crop improvement. in Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore

5. Tulsi Naik KS, A Ramesha, MS Ranjini, Vanitha C, Chandana, KM Ponnuvel, Manthira Moorthy (2024) Improved silkworm hybrids resistant to Bombyx mori Bidsenovirus (BmBDV) developed through molecular marker assisted breeding in Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore
6. Pawan Shukla, Harshitha Prakash, A Ramesha, Gondi S Arunakumar, S Gandhi Doss, KM Ponnuvel, S. Manthira Moorthy (2024) Identification of suitable reference genes for accurate normalization of qPCR data under biotic stresses in Mulberry (*Morus indica* L.) in Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore
7. ए. रमेशा, हिमांशु दुबे, मनुदीप राव, पवन शुक्ला, के.एम. पोन्नूवेल, के. सुरेश, मंदिरा मूर्ति (2024) शहतूत में पाउडरी मिल्ड्यू रोग की संवेदनशीलता में शामिल एमएलओ जीन और उनके आनुवंशिक वेरिएंट की पहचान नामक शीर्षक के साथ रे. जै. प्रौ. अ. प्र. द्वारा दिनांक 15.02.2025 को आयोजित राष्ट्रीय राजभाषा तकनीकी सेमिनार के स्मारिका सह शोध पत्र संकलन में ISBN No. 978-81-967880-7-0 के साथ प्रकाशित हुआ।
8. पवन शुक्ला*, ए. रमेश, जी. सुब्रह्मण्यम एवं एस. मंदिरा मूर्ति (2024) शहतूत (मोरस इंडिका एल) में जैविक तनाव के दौरान क्यूपीसीआर डेटा के सटीक विश्लेषण के लिए संदर्भ जीन की उपयोगिता नामक शीर्षक के साथ रे. जै. प्रौ. अ. प्र. द्वारा दिनांक 15.02.2024 को आयोजित राष्ट्रीय राजभाषा तकनीकी सेमिनार के स्मारिका सह शोध पत्र संकलन में ISBN No. 978-81-967880-7-0 के साथ प्रकाशित हुआ।
9. हिमांशु दुबे*, इंदुमती, कामाची बी., राजल देबनाथ, ए. रमेश, एस. मंदिरा मूर्ति, के. एम. पोन्नूवेल, एवं कल्लारे पी अरुण कुमार (2024) भारतीय सुनहरे रेशम कीट, एंथेरिया असामेन्सिस हेल्फर (लेपिडोप्टेरा, सैटर्निडे) के जीनोम का अनुक्रमण एवं संयोजन नामक शीर्षक के साथ रे. जै. प्रौ. अ. प्र. द्वारा दिनांक 15.02.2024 को आयोजित राष्ट्रीय राजभाषा तकनीकी सेमिनार के स्मारिका सह शोध पत्र संकलन में ISBN No. 978-81-967880-7-0 के साथ प्रकाशित हुआ।
10. इंदुमती कामाची. बी., हिमांशु दुबे*, एस. मंदिरा मूर्ति, जी. सुब्रह्मण्यम, रजल देबनाथ, विक्रम कुमार अभिषेक सिंह (2024) एंथेरिया असामेन्सिस (मुगा) और अन्य सैटर्निड्स सिल्कमॉथ में एटैसिन जीन परिवार की जीनोम व्यापक पहचान, लक्षण वर्णन और तुलनात्मक विश्लेषण नामक शीर्षक के साथ रे. जै. प्रौ. अ. प्र. द्वारा दिनांक 15.02.2024 को आयोजित राष्ट्रीय राजभाषा तकनीकी सेमिनार के स्मारिका सह शोध पत्र संकलन में ISBN No. 978-81-967880-7-0 के साथ प्रकाशित हुआ।
11. हर्षिता प्रकाश, पवन शुक्ला, के. एम. पोन्नूवेल, एवं एस. मंदिरा मूर्ति (2024) पिंक मीलीबग, मैकोनेलिकॉक्स हिर्सुटस (ग्रीन) की आंतरिक शारीरिक संरचना का अध्ययन नामक शीर्षक के साथ रे. जै. प्रौ. अ. प्र. द्वारा दिनांक 15.02.2024 को आयोजित राष्ट्रीय राजभाषा तकनीकी सेमिनार के स्मारिका सह शोध पत्र संकलन में ISBN No. 978-81-967880-7-0 के साथ प्रकाशित हुआ।

12. तुलसी नायक के. एस., वनिता सी., संध्या रसलकर, रवि कुमार जी, उपेंद्र नोंगथोंबा, एस. मंदिरा मूर्ति (2024) नोसेमा बॉम्बिसिस के विरुद्ध बोम्बक्स मोरी के लैमेरिन नस्ल द्वारा प्रदर्शित सहनशीलता से जुड़े आणविक क्रियाविधि का अध्ययन नामक शीर्षक के साथ रे. जै. प्रौ. अ. प्र. द्वारा दिनांक 15.02.2024 को आयोजित राष्ट्रीय राजभाषा तकनीकी सेमिनार के स्मारिका सह शोध पत्र संकलन में ISBN No. 978-81-967880-7-0 के साथ प्रकाशित हुआ।
13. जी. सुब्रमण्यम, एन. राजीव भरत, दीपिका अयंगर, पवन शुक्ला, के.एम. पोन्नुवेल, एस. मंदिरा मूर्ति (2024) वन्य रेशमकीटों में रोगजनकों का पता लगाने के लिए मल्टीप्लेक्स पीसीआर उपकरण का विकास नामक शीर्षक के साथ रे. जै. प्रौ. अ. प्र. द्वारा दिनांक 15.02.2024 को आयोजित राष्ट्रीय राजभाषा तकनीकी सेमिनार के स्मारिका सह शोध पत्र संकलन में ISBN No. 978-81-967880-7-0 के साथ प्रकाशित हुआ।

राजभाषा कार्यान्वयन- OFFICAL 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 14 से 30 सितंबर 2023 तक मनाया गया और विभिन्न कार्यक्रम आयोजित किए गए।
Hindi Pakhwada was celebrated from 14th-30th September 2023 by conducting various events.

PUBLICATIONS

1. Deepika I., Ramesh K.V., Kumar I., Singh A., Debnath R., Dubey H., Shukla P., Ponnuvel K.M., Moorthy S.M. and Subrahmanyam, G., (2024) Molecular diagnostics in sericulture: A paradigm shift towards disease diagnosis in silkworms. *Entomologia Experimentalis et Applicata*, 172(5), pp.372-382.
2. Harshitha Prakash, Pawan Shukla, A. Ramesha, Gondi S. Arunakumar, S. Gandhi Doss, and Kangayam M. Ponnuvel(2023) Evaluation of reference genes for accurate normalization of qPCR data under biotic stresses in mulberry (*Morus indica L.*). *Scientia Horticulturae* 323: 112507
3. Subrahmanyam, G., Ponnuvel, K.M., Arunkumar, K.P., Rahul, K., Moorthy, S.M. and Sivaprasad, V., (2023) Molecular methods for diagnosis of microbial pathogens in muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae). *International Journal of Industrial Entomology*, 47(1).

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11. Pawan Shukla, Anirudh Kumar, and Rakesh Kumar (2023) Climate change and stress mitigation strategy in plants." Frontiers in Plant Science 14: 1291905.
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Books/Book chapters

1. Training Manual on Molecular Techniques Applied in the Field of Sericulture – II Fundamentals and Protocols ISBN No 978-81967880-5-6
2. K S Tulsi Naik, Pawan Shukla, Harshitha Prakash, Vanitha C (2023) Genomic DNA isolation from Silkworm and host plant, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 1-3

3. Gangavarapu Subrahmanyam, Deepika Iyyengar (2023) Isolation of RNA and cDNA synthesis from Silkworm, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg 4-6
4. Himanshu Dubey, Rajal Debnath, Indumathi Kamatchi (2023) PCR and qRT-PCR, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 7-13
5. Rajal Debnath, Himanshu Dubey, Lavanya C (2023) Barcoding of insects, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 14-18
6. K S Tulsi Naik(2023) Transkingdom RNA interference (TKRNAi) in silkworms, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 19-20
7. K S Tulsi Naik, A Ramesha (2023) Marker Assisted selection (MAS) in crop improvement, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 21-23
8. Kangayam M. Pounnuvel, and Gangavarapu Subrahmanyam (2023) Molecular diagnosis of pebrine disease in silkworms, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 24-27
9. Harshitha Prakash, and Pawan Shukla (2023) Micro-injection, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 28-30
10. Ramesha A, Pawan Shukla, Harshitha Prakash (2023) Plant tissue culture in mulberry crop improvement, In Training Manual on Molecular Techniques applied in the field of Sericulture-II Published by SBRL, ISBN No 978-81967880-5-6 pg No 31-35

Popular Articles

1. Pawan Shukla, Harshitha Prakash, ChannabasavaVeershetty, Kangayam M. Ponnuvel (2023) Broad mite *Polyphagotarsonemus latus* (Banks): A devastating pest for mulberry. Indian Silk- November-December Vol.14 (Old 62) No.5, Page 8-9

Technical Brochure

1. Point -of-care Diagnostic technology for detection of *Nosema* Sps. in silkworms
2. Improved bivoltine double hybrid (FC1RXFC2R) resistant to viral Falcherie (BmBDV/DNV2)

AWARDS

1. Dr. G. Subrahmanyam, Scientist C received the best oral presentation award in the Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore
2. Dr. Pawan Shukla, Scientist C received the best Poster presentation award in the Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore

3. Dr. Himanshu Dubey, Scientist C received the best oral presentation award (third Place) in the Silkworm SEEDCON 2024; an International Conference on Silkworm Seed Industry: Opportunities and Future Prospects held organized by Central Silk Board on 30th-31st January 2024 at Bangalore
4. Dr. Himanshu Dubey, Scientist C received the best oral presentation award (third Place) in the राष्ट्रीय राजभाषा तकनीकी सेमिनार organized by SBRL, Kodathi on 15th February 2024
5. Dr. K.S. Tulsi Naik, Scientist C received best oral presentation award (Vanya theme) in the राष्ट्रीय राज भाषा तकनीकी सेमिनार organized by SBRL, Kodathi on 15th February 2024

***Bombyx mori* Breeds Registration**

As per the Germplasm Registration committee meeting held on 01.02.2024, the committee has approved for the registration of SK6BR and SK7BR *Bombyx mori* Silkworm breeds resistant to Viral Flacherie (DNV2/BmBDV) developed through Molecular marker assisted breeding for BmBDV resistance at Seri Biotech Research Laboratory Bangalore, in collaboration with CSB-CSRTI, Berhampore. (CSB/CSGRC/GRC/5(118)/MUL/TECH/2023-24 Dated 17.02.2024)

RESEARCH ADVISORY COMMITTEE

S.N.	Details of Committee	Designation
1	Dr. N. K. Krishnakumar, Retired Deputy Director General (Horticulture) Indian Council of Agricultural Research (ICAR) New Delhi.	Chairperson
2	Dr. Sanjay Ghosh Associate Professor, Institute of Bioinformatics and Applied Biotechnology Bangalore.	Member
3	Dr. M. Mohan Principal Scientist, Division of Genomic Resources, NBAIR, Bangalore.	Member
4	Dr. Nataraj Karaba Professor, Dept. of Crop Physiology, UAS, GKVK, Bangalore.	Member
5	Dr. K. Vijayan, Retired Scientist, Central Silk Board	Member
6	The Director Central Sericulture Research & Training Institute, Mysore.	Member
7	The Director Central Sericulture Gemplasm Resources Centre, Hosur.	Member
8	The Director Seri-Biotech Research Laboratory, Kodathi, Carmelram post, Sarjapur Road, Bangalore.	Member-Convener

HUMAN RESOURCES

S.N.	Name	Designation
1	Dr. S. Manthira Moorthy	Director (w.e.f. 01.12.2023)
2	Dr. K. M. Ponnuvel	Scientist- D & Officer In-charge (From 01.06.2022 to 31.05.2023)
3	Dr. G. Subrahmanyam	Scientist- C and Officer In-charge (From 01.06.2023 to 30.11.2023)
4	Dr A. Ramesha	Scientist- C
5	Dr. Pawan Shukla	Scientist- C
6	Dr. K. S. Tulsi Naik	Scientist- C
7	Dr. Rajal Debnath	Scientist- C
8	Dr. Himanshu Dubey	Scientist-C
9	Mr. R. N. Srikantaiah	Senior Technical Assistant
9	Mr. G. Raghavendar	Field Assistant
10	Mr. Srinivas Raju K.V.	Field Assistant

Research fellows / Assistants

S.N.	Name	Designation
1.	Ms. Indumathi Kamatchi	Ph.D. Scholar & Junior Research Fellow
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

Administration

S.N.	Name	Designation
1	Ms. Manjula S.	Superintendent
2	Kenchappa	Multi-tasking, Staff

SUPERANNUATION



Mr. K. Nanjundappa, Staff Car Driver and E. Shivanna, SFW (TS) retired from service of Central Silk Board (Government of India) on attaining superannuation. The SBRL fraternity thanks them for their esteemed service and wish them happy retired life.

OTHER ACTIVITIES

Hindi Fortnight

The Hindi Fortnight was observed from 14th-30th September 2023, featuring Hindi Day and a variety of competitions to celebrate the occasion. Additionally, Silk Day was commemorated on 20th September 2023, honoring the visionary Dr. Shyama Prasad Mukherjee for his significant contributions to the development of India's silk industry.

National Unity Day

The National Unity Day was observed on 31st October 2023 in which staff of SBRL have taken pledge.

Vigilance Awareness Week

Vigilance awareness week was observed w.e.f. 30th Oct - 05th Nov 2023

World Environment Day

World environment day was observed on 06th June 2023. On this occasion, plantation drive and

Yoga Day

Yoga day was organized on 21st June 2023 in which all the staff of SBRL were actively participated

Health Camp

A health camp was organized on 12.12.2023 in which Doctor and his staff from Primary Government Hospital, Kodathi were invited for the general check-up and blood test for all the staff/students of SBRL and SSTL.