# वार्षिक प्रतिवेदन
# Annual Report
## 2021-2022

**रेशम जैव-प्रौद्योगिकी अनुसंधान प्रयोगशाला**
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# ANNUAL REPORT 2021-22


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| Published by | : | **Dr. V. Sivaprasad**<br>Director<br>Seri-Biotech Research Laboratory<br>Bengaluru - 560 035 |
| Editorial Committee | : | Dr. Pawan Shukla<br>Dr. K. S. Tulsi Naik<br>Dr. A. Ramesha<br>Dr. Rajal Debnath<br>Dr. G. Subrahmanyam<br>Dr. Himanshu Dubey<br>Dr. K. M. Ponnuvel |
| Hindi Translation | : | Dr. Pawan Shukla |
| Cover Page Design | : | Dr. Rajal Debnath |
| No. of Copies | : | 75 copies |
| Printed at | : | M/s Sharadh Enterprises,<br># 51, Car Street,<br>Halasuru,<br>Bangalore-560008 |


## COVER PAGE

The cover page shows the protein structural model of RNA-dependent RNA polymerase (RdRp) from Cypovirus infecting *Antheraea assamensis* Helfer. The model is generated using Modeller v10.2

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# प्राक्कथन

मुझे वर्ष 2021-22 के लिए रेशम जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला, केन्द्रीय रेशम बोर्ड, कोड़ती की वार्षिक रिपोर्ट प्रस्तुत करते हुए अत्यंत प्रसन्नता हो रही है। यह वर्ष चल रही अनुसंधान परियोजनाओं के उत्साहजनक परिणामों और अगले वर्ष में किए जाने वाले उज्ज्वल नए प्रस्तावों के साथ एक और सफल वर्ष था। जबकि आम जनता कोविड-19 महामारी के बाद सामान्य स्थिति में लौट आई है, रेशम जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला में अनुसंधान एवं विकास कार्य वन्या और शहतूत दोनों क्षेत्रों में रोमांचक परिणाम नियमित रूप से देते रहे है।

निरंतर अनुसंधान प्रयासों से ए. मायलिट्टा को संक्रमित करने वाले इफ्लावायरस के रोग की एटियलजि और महामारी विज्ञान को समझने में महत्वपूर्ण परिणाम प्राप्त हुए हैं, जो क्षेत्र स्तर पर संक्रमण का मुकाबला करने में फायदेमंद हो सकता है; मूगा रेशमकीटों में वाइरोसिस के कारक एजेंट के रूप में साइपोवायरस की पहचान हुई है, जिससे बेहतर रोग प्रबंधन किया जा सकता है और रोग प्रतिरोधी ए. असामेंसिस नस्लों का विकास भी हो सकता है; पेब्राइन का पता लगाने के लिए टैकमैन टेस्ट विकसित किया गया है, जिसमें उच्च संवेदनशीलता और सटीकता के साथ कई रोगजनकों का पता लगाने की अपार संभावनाएं है; एमएलओ जीन की पहचान हुई है, जिनकी शहतूत में चूर्णिल आसिता रोग प्रतिरोधक क्षमता/संवेदनशीलता में भूमिका वर्तमान में समझी जा रही है; और देश भर में न्यूक्लियोपॉलीहेड्रोसिस वायरस (एनपीवी) के प्रति प्रतिरोधी एमएएसएन 4 संकरों का बड़े पैमाने पर सत्यापन किया जा रहा है।

नवीनतम पहलुओं में बीज गुणन और उत्पादन केंद्रों पर पेब्राइन का पता लगाने के लिए “लेटरल फ्लो एसे” (एलएफए) शामिल है, जो औद्योगिक साझेदार के सहयोग से “प्वाइंट ऑफ केयर” (पीओसी) परीक्षण देने का वादा करता है। एलएफए तकनीक एक समय में कई बीमारियों के लिए किट विकसित करने का भी वादा करती है। अन्य शोधों में इरी और मूगा रेशमकीट में नई नस्लों और संकरों को विकसित करने के लिए है, जिसमें मूगा रेशमकीट के कार्यात्मक जीनोमिक्स और शहतूत में “टुकरा रोग” के लिए सर्वोत्तम प्रबंधन रणनीति तैयार करने के लिए मिलीबग स्राव का दोहन करना शामिल है।

व्यापक तौर पर, चालू वर्ष के दौरान एस.बी.आर.एल. की प्रगति विशेष रूप से वन्या रेशम उत्पादन के लिए अज्ञात को समझने में अधिक उल्लेखनीय रही है। इसके अलावा, अनुसंधान विविधीकरण से और अधिक अत्याधुनिक प्रौद्योगिकी/उपकरण रेशम उद्योग के हितधारकों के लिए लाभकारी हो सकते हैं। परिवर्तित दृष्टिकोण अन्य सेरी-आर एंड डी संस्थानों के साथ समन्वय करने के लिए भविष्य में सार्थक सहयोग और समस्या समाधान के लिए मार्ग प्रशस्त कर रहा है। हमेशा की तरह, वर्ष के दौरान वैज्ञानिक प्रकाशन फिर से उत्कृष्ट गुणवत्ता के थे।

एस.बी.आर.एल. अनुसंधान एवं विकास गतिविधियों को लागू करने के लिए केंद्रीय रेशम बोर्ड के सदस्य सचिव और निदेशक (वित्त) के निरंतर समर्थन के लिए बहुत आभारी है। विशेषज्ञ मार्गदर्शन के लिए अनुसंधान सलाहकार समिति और वित्तीय समर्थन के लिए विभिन्न प्रायोजक एजेंसियों (डीबीटी/डीएसटी/स्वीडिश अनुसंधान परिषद) का आभारी हूँ। मैं सहयोगी भागीदारों के साथ-साथ एस.बी.आर.एल. के वैज्ञानिकों के योगदान की भी सराहना और अभिस्वीकृति करना चाहूंगा।

मुझे विश्वास है कि पाठकों को यह अंक बहुत उपयोगी और ज्ञानवर्धक लगेगा।

**डॉ. वी. शिवप्रसाद**
निदेशक

# FOREWORD

It gives me immense pleasure to present the Annual Report of Seri-Biotech Research Laboratory, Central Silk Board, Kodathi for the year 2021-22. This was just another fruitful year with encouraging results from the ongoing research projects and bright new proposals to be undertaken in the next year. Whilst the general population returns to normalcy following the COVID-19 pandemic, R&D works at SBRL remained steady delivering the exciting outcomes in both vanya and mulberry sectors.

The continuous research efforts have yielded the significant outcomes in deciphering disease etiology and epidemiology of Iflavirus infecting *Antheraea mylitta,* which could be beneficial in combating the infections at the field level; identification of cypovirus as causative agent for virosis in muga silkworms, which could lead to better disease management and also the development of disease resistant *Antheraea assamensis* breeds; TaqMan assay for the detection of pebrine, which throws open possibilities of detecting multiple pathogens with higher sensitivity and accuracy; identification of MLO genes, whose role in mulberry powdery mildew disease resistance/susceptibility is being deciphered currently; and large scale validation of MASN4 hybrids tolerant to nuclear polyhedrosis across the country.

The latest initiatives include "Lateral Flow Assay" (LFA) for the detection of pebrine at seed multiplication and production centres in collaboration with industrial partner promises to deliver "Point of Care" (POC) testing. The LFA technology also holds promise to develop kits for multiple diseases at a time. The other researches are in eri and muga silkworms to develop new breeds and hybrids including the functional genomics of muga silkworm and exploiting the mealybug secretions for devising best management strategies for "Tukra disease" in mulberry.

Overall, the progress of SBRL during the current year has been more remarkable for deciphering the unknowns especially in vanya sericulture as well. In addition, the research diversification may lead to more cutting-edge technology/tools for the benefit of stakeholders of silk industry. The changed approach to coordinate with the other Seri-R&D Institutes is paving way for future meaningful collaborations. As usual, the scientific publications during the year were again of outstanding quality.

SBRL is highly grateful to the Member Secretary and Director (Finance) of Central Silk Board for their constant support to implement R&D activities. Sincere gratitude is due to the Research Advisory Committee for expert guidance and different sponsoring agencies (DBT/DST/Swedish Research Council) for their financial support. I would also like to appreciate and acknowledge the contributions of collaborating partners as well Scientists at SBRL.

I am sure that this volume will be very useful and informative.

**Dr. V. Sivaprasad**

DIRECTOR

# VISION AND MISSION

## 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 silk biotechnology and silk- based biomaterials towards improvement in silk productivity
- To interact with reputed R&D institutions in sericulture and allied activities
- To develop and disseminate technology to other R&D organizations

## OBJECTIVES

- Conduct scientific research in frontier areas of modern biology for developing potential applications in improving silk productivity
- Conduct research on silk for biomaterial and biomedical applications and by-product utilization
- Development and patenting of products/technologies
- Capacity building in Seri-biotechnology
- Strengthening institutional framework to support research programmes
- Publication of R&D outcome
- Collaborative research programmes with other R&D organizations in India and abroad including industry
- Efficient functioning through RFD System
- Improving internal efficiency / responsiveness / service delivery of the institute
- Training for employable manpower development

## FUNCTIONS

- To formulate and implement research projects in frontier areas of modern biology
- To take up collaborative projects with other institutions in applied research
- To develop and disseminate the products/technologies
- To generate Human Resource in Seri-biotechnology

# अनुसंधान के मुख्य आकर्षण

1. मूंगा रेशमकीट में विषाणु रोग के लिए उत्तरदायी रोगजनक की पहचान साइपोवायरस-4 (रेओविरिडे) के रूप में की गई है ।

2. बैकुलोवायरस और इफ्लावायरस संक्रमण की महामारी-विज्ञान क्रमशः ए. प्रोयली और ए. मायलिट्टा में स्थापित किया गया है।

3. *बी. मोरी* में माइक्रोस्पोरिडियन रोगजनकों का शीघ्र पता लगाने के लिए टैकमैन टेस्ट विकसित किया गया है ।

4. एल.एफ.ए. के माध्यम से *एन. असामीस* और *एन. मायलिट्टा* का जल्द पता लगाने के लिए उपयुक्त स्पोर वाल प्रोटीन के खिलाफ एंटीबॉडी को बनाया गया है।

5. गैर-कार्यात्मक (non-functional) उत्परिवर्तन और उनके अभिव्यक्ति विश्लेषण के लिए एमएलओ 2 और एमएलओ 6 ए जीन के लिए चूर्णिल आसिता रोग के लिए शहतूत जीनोटाइप सहिष्णुता की जांच की गई।

6. मार्कर असिस्टेड चयन के माध्यम से सीएसआर-2 और सीएसआर-27 की बाइडेन्सोवायरस (डीएनवी-2) प्रतिरोधी समयुग्मजी लाइनें विकसित की गई है ।

# RESEARCH HIGHLIGHTS

1. Pathogen responsible for virosis disease in muga silkworm was identified as cypovirus-4 (Reoviridae).
2. Epidemiology of baculovirus and iflavirus infections in *Antheraea proylei* and *Antheraea mylitta* respectively was established.
3. TaqMan assay has been developed for early detection of microsporidian pathogen in *Bombyx mori*.
4. Antibodies were raised against spore wall proteins suitable for early detection of *Nosema assamensis* and *Nosema mylitta* through LFA.
5. Mulberry genotypes tolerance to powdery mildew disease was screened for MLO2 and MLO6A gene for non-functional mutations and their expression analysis.
6. Developed BmBDV (DNV2) resistant homozygous lines of CSR2 and CSR27 through marker assisted selection.

# LIST OF RESEARCH PROJECTS

## ON-GOING RESEARCH PROJECTS

1. **PRP-08002MI** : Identification of powdery mildew resistant genes and validation of CAPS marker for Chalcone synthase in mulberry spp (May 2019 - May 2022)
2. **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 - July 2022); **DBT Funded**
3. **PIT-08004MI** : Study on epigenetic and autophagy modifiers on induction of haploid microspore embryogenesis in mulberry (March 2020 - February 2023)
4. **AIT-08005MI** : Development and evaluation of Bidensovirus resistant silkworm hybrids developed from marker assisted breeding lines - Phase II (March 2020- February 2023)
5. **AIT-08006 EF** : Development of lateral flow assay (LFA) kit for diagnosis of pebrine disease in silkworms (March 2021 - January 2023), **DBT-BIRAC Funded**
6. **ARP-08007MI** : Biological and molecular characterization of virosis in Muga silkworm, (*Antheraea assamensis* Helfer) (March 2022- February 2025)
7. **AIB-08008MI** : Development and Evaluation of Eri silkworm (*Samia ricini* Donovan) breeds/ hybrids with improved productivity (March 2022- February 2025)
8. **ARP-08001CI** : Studies on the genetic characterization, transmission and tissue distribution of Iflavirus infecting the Indian tropical tasar silkworm, *Antheraea mylitta* (April 2018 – September 2022); **Swedish Research Council Funded**

# PROGRESS OF ONGOING RESEARCH PROJECTS

## 1. PRP08002MI: Identification of powdery mildew resistant genes and validation of CAPS marker for Chalcone synthase in mulberry spp.

(In collaboration with CSR&TI, Berhampore; **Duration:** May 2019- May 2022)

A. Ramesha, Himanshu Dubey; K. Suresh*; K.Vijayan# (Up to May 2021)

*CSR&TI, Berhampore; #CSB, Bangalore

### Objectives:

1. To identify powdery mildew susceptibility genes Mildew Resistance Locus O (*MLO*) from mulberry
2. To screen powdery mildew resistant mulberry genotypes for an association of non-functional mutation in the candidate *MLO* gene with disease resistance
3. Validation of CAPS marker for chalcone synthase gene involved in powdery mildew resistance in diverse germplasm accessions / segregating progenies

Powdery mildew is one of the major foliar diseases, which negatively affect quality and quantity of mulberry leaf available to produce silk. Development of resistant/tolerant varieties forms an important approach for management of the disease. For disease resistance breeding, deployment of susceptibility (S) genes offers alternative approach to Resistance (R) genes as formerly confer broad spectrum and durable resistance. Mildew resistance Locus O (MLO) based resistance successfully being utilized in commercial plant breeding in few crops to counter the powdery mildew fungi infection. Therefore, in this project we aim to identify MLO genes associated with powdery mildew susceptibility in mulberry and identify non-functional mutations in the MLO genes with resistance phenotype in different genotypes.

Genome wide analysis identified 16 *MLO* genes from mulberry genome. Combined analysis of phylogenetic tree, conserved motif analysis and gene expression identified *MLO2* and *MLO6A* as potential candidate genes involved in powdery mildew susceptibility in mulberry.

*MLO* genes are susceptibility (S) genes, genotypes expressing low levels of *MLO*2 and *MLO6A* genes or having non-functional mutations are expected to be resistant to powdery mildew disease. Therefore, we analysed the expression of *MLO* genes and also checked for the non-functional mutations in the powdery mildew resistant mulberry genotypes. *MLO2* and *MLO6A* expression level were checked in different genotypes resistant to powdery mildew disease after spraying $10^5$ fungal spores/ml (**Fig.1**).

The non-functional mutations in the *MLO2* and *MLO6A* genes were checked after cloning and sequencing the coding sequences from different resistant genotypes for powdery mildew disease. The coding sequence of *MLO2* from ME-0260 was analysed with the reference *MLO2* genomic and coding sequence, it was found that intron 4 is retained in the coding sequence of ME-0260 (**Fig. 2**). Also, the translated protein is truncated as there is a stop codon in intron

4 which was retained in the coding sequence of ME-0260. The coding sequence of *MLO2* in TL-1 (Thailand lobed-1) variety compared with reference *MLO2*, It was observed that in TL-1 intron 9 and intron 10 were retained and exons 12, 13 and 14 were skipped (**Fig. 3**).

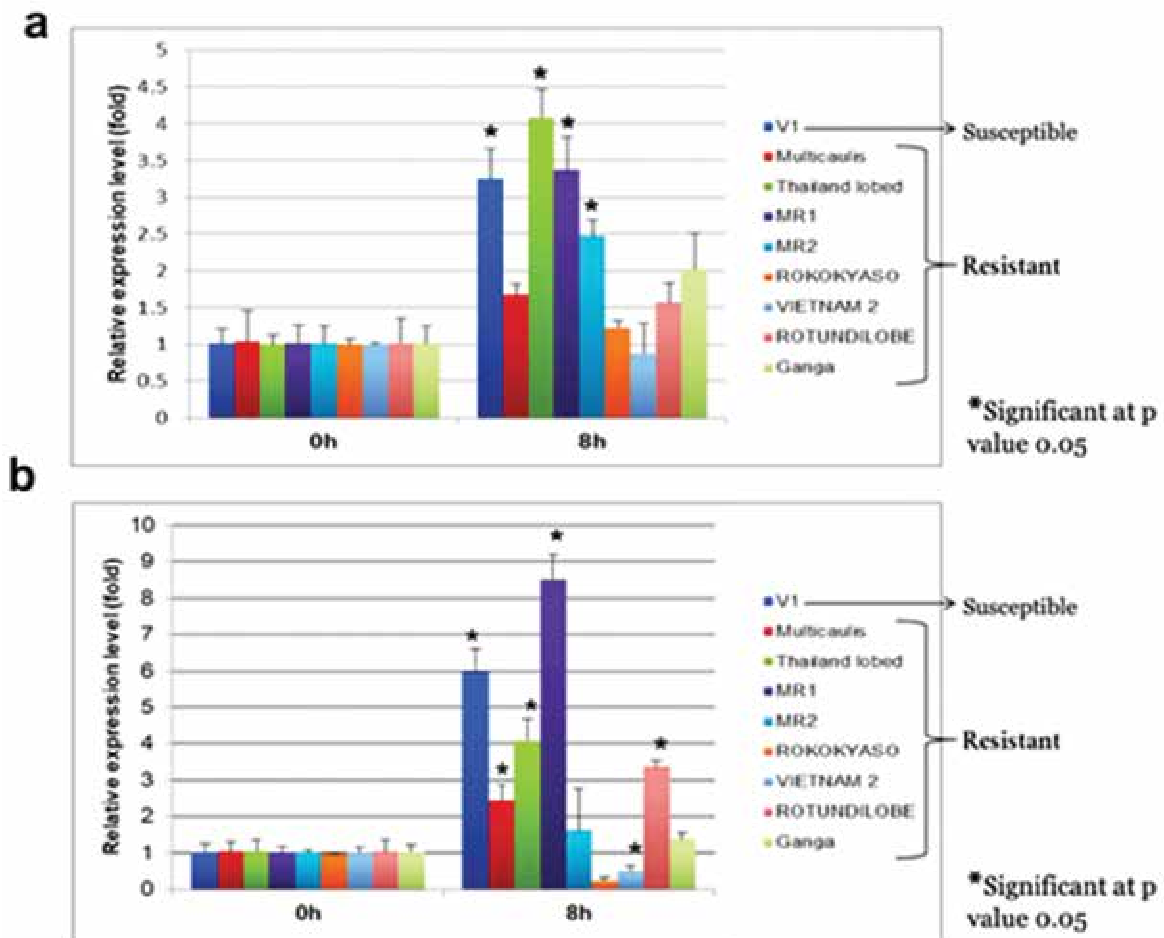
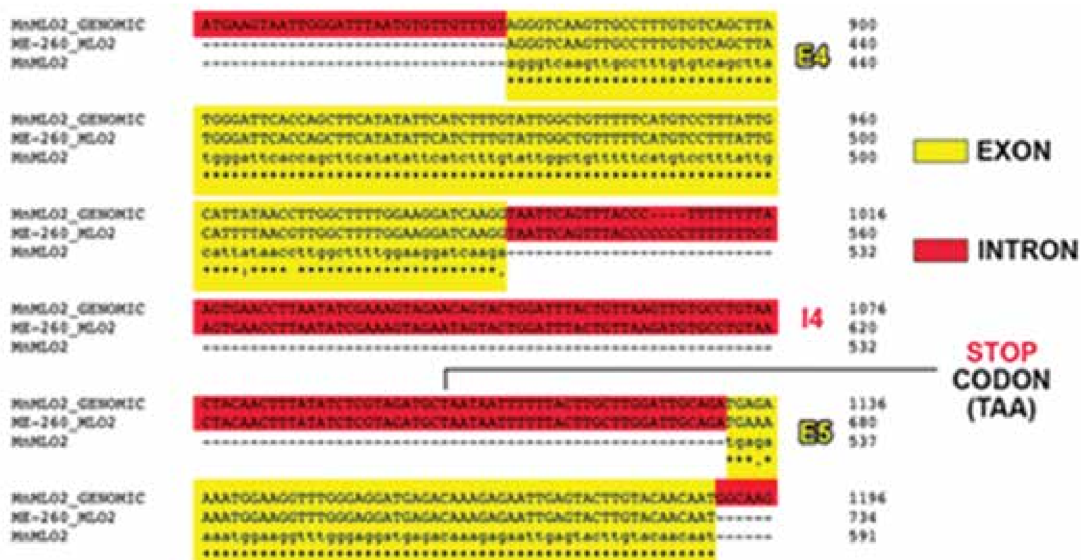



**Figure 1**: **a)** MLO2 gene expression; **b)** MLO6A gene expression in susceptible and resistant genotypes at 0h and 8h after powdery mildew infection.



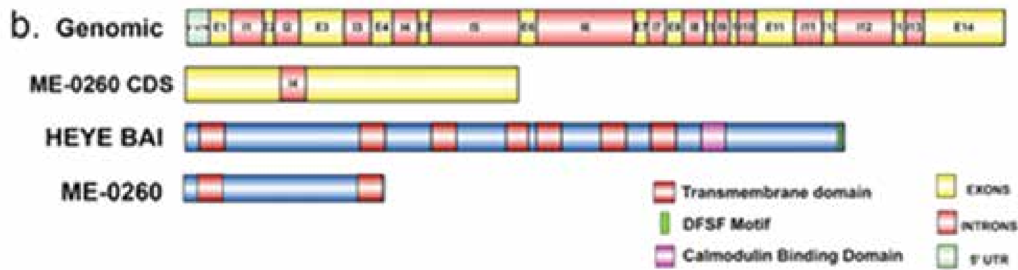



**Figure 2: a)** Multiple sequence alignment of the coding sequence of *MLO2* from ME-0260 with *MnMLO2* coding sequence, **b)** Schematic representation of the *MLO2* gene, CDS and protein structures from Heyebai and ME-0260 genotypes.

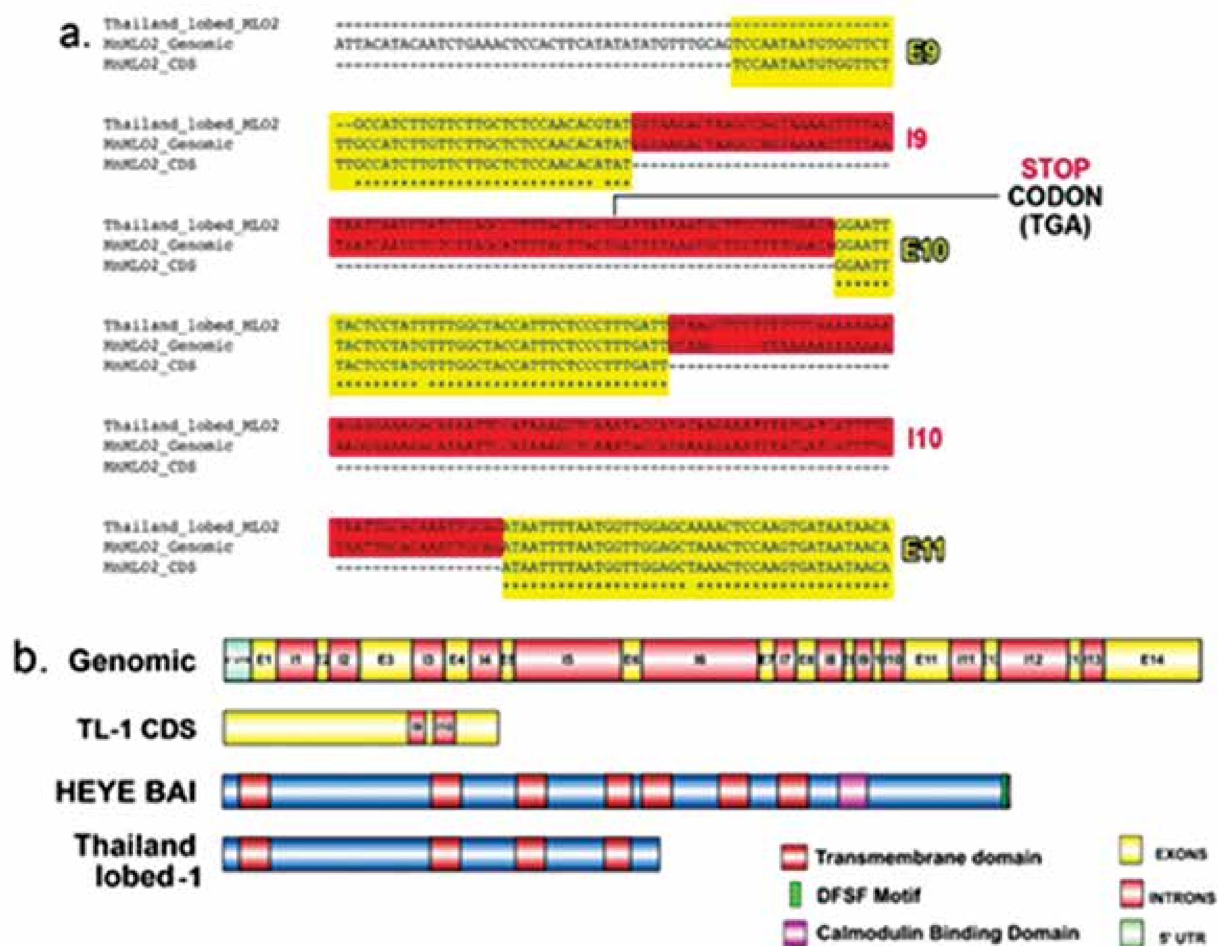



**Figure 3**: **a)** Multiple sequence alignment of the coding sequence of *MLO2* from Thailand Lobed with *MnMLO2* coding sequence; **b)** Schematic representation of the *MLO2* gene, CDS and protein structures from Heyebai and Thailand Lobed genotypes

Because of the retention of the 9th intron, a premature stop codon (TGA) was generated and that resulted in truncated MLO2 protein in the TL-1 genotype. Alternative splicing of *MLO2* gene may be playing a regulatory role in conferring powdery mildew resistance in ME-0260 and Thailand lobed genotypes.

## 2. **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 July 2022)

Ravi Kumar G[$], Tulsi Naik K S, Upendra Nongthomba[*], K. Vijayan[#]
[$]CSGRC, Hosur; [*]IISc, Bangalore, [#]CSB, 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 Microsporidian infection also known as pebrine infection is one of the major threats to sericulture industryand in *Bombyx mori* (*B.mori*). The causative pathogen identified as *Nosema bombycis*. These Microsporidians are among the smallest and most primitive of eukaryotic cells, representing one of the earliest branches of the eukaryotic phylogenetic tree. The spore is the most visible sign of infection by microsporidia and its structure, texture and nature are the characteristic feature of microsporidia. Among numerous silkworm, *B. mori* breeds, the CSR2 is a commercial productive silkworm breed, which is highly susceptible to microsporidian infections. However, there are also reports about the "**Lamerin" or "Leimaren**", breed of silkworm *B. mori* that exhibited most resistance/tolerance to microsporidians is commercially exploited breed that belongs to Manipur, North East India. It is also the only breed of the silkworm *B. mori* which survives with microsporidian infections (Bhat et al 2005).

### Microsporidian infection and differential gene expression analysis

The freshly molt third instar larvae (Day 1) of CSR2 and Lamerin breeds was starved for six hours and then fed mulberry leaves smeared with $1 \times 10^5$ spores/ml of highly virulent *Nosema bombycis* spores. The control groups fed with mulberry leaves smeared with sterile water. After infection, larvae were subsequently fed with normal mulberry leaves till spinning. Approximately 10-12 larvae were collected at each time points starting from 12, 24, 48, 72 and 96 hours post infection, for genomic DNA and total RNA isolation. The larval midguts was dissected out, washed in DEPC treated water, and suspend in Trizol reagent (Invitrogen) and keep at -80$^0$C. Total RNA was extracted using Qiagen RNA extraction kits for both, according to the manufacturer's protocol. The purity of all RNA samples will be 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.

## Confirmation of the microsporidia infection in the midgut samples

The genomic DNA isolated from the infected midgut samples at different time points was PCR amplified with the β-tubulin primers to check the pebrine infection through conventional PCR. The conventional PCR results did not shown any infection at 12 hpi, however the infection could be observed after 24hpi using Nosema gene specific primers. The infection was also confirmed using other nosema specific genes like SWP5, PTP1 and PTP 2, all the gene specific primers showed amplification from 24 hpi. It was also interesting to observe pebrine infection even in the Lamerin infected midgut samples staring from 24 hpi. In the earlier, bioassay experiments the pebrine infection was given at $5^{th}$ instar therefore, the survivability/mortality between both the breeds could not be evaluated. Therefore, in this experiment, the infection was given as early as $3^{rd}$ instar so that some of the larvae from both the infected and control of both the breeds could be continued till the cocoon formation to check the survivability and visual symptoms of the pebrine infection. The results clearly indicated the infection of *Nosema bombycis* in both CSR2 and Lamerin breeds (**Fig 4**).

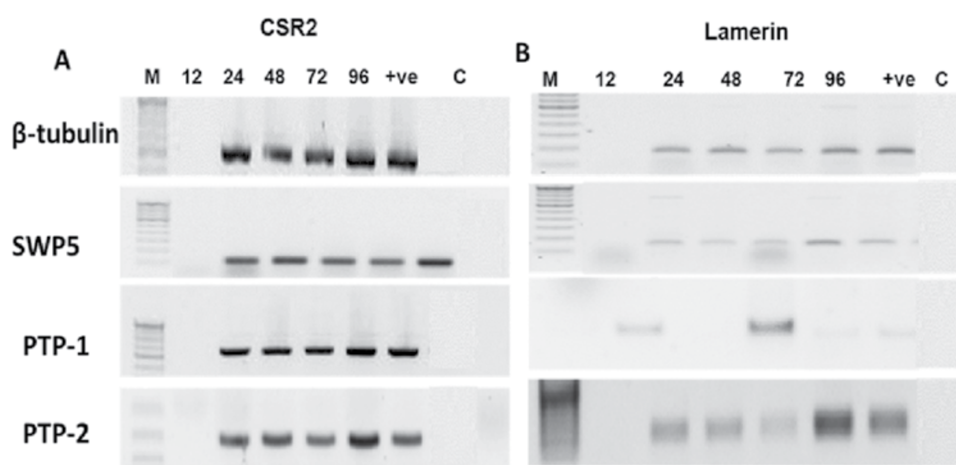



**Figure 4:** PCR analysis *N.* bombycis infected midgut tissue DNA of CSR2 (A) and Lamerin (B) with different Nosema specific primers. The genomic DNA of orally infected 3rd instar larvae were collected at different time points starting at 12, 24, 48, 72, 96, hours post infection (hpi).

## Differential gene expression studies for *N.bombycis* and host immune specific genes.

The qPCR analysis was carried out to check the differential expression of Nosema specific and host immune specific genes to study the gene expression patterns in the midgut of CSR2 (susceptible) and Lamerin (tolerant/resistant) infected with the microsporidian *N. bombysis*. The qPCR analysis showed significantly increased expression of all the Nosema specific genes from 12-96hpi in CSR2 compared to lamerin. In Lamerin breed thoughinitially there is increase in expression of Nosema genes from 12-24hpi subsequently there was no significant increase in gene expression across time points. Further, the qPCR analysis showed significantly increased expression of *Serpin3* gene starting from 12hpi to 96hpi in

Lamerin, while in CSR2 initially *Serpin 3* expression was increased from 12hpi till 48hpi but gradually the expression was decreased. In contrast the *Serpin4* gene increased significantly at 96hpi, however in CSR2 only at 12hpi there was expression of *Serpin 4* but decreased gradually at different time points. Except for these two genes other antimicrobial proteins, i.e., *Gloverins*, *Cecropins*, & *Moricins* that are specifically expressed due to *N. bombycis* infection were significantly upregulated initially 12-48hpi in CSR2 but gradually decreased during 72-96hpi. While in Lamerin breeds there was significantly higher expression of antimicrobial proteins genes from the initial 12hpi till 96hpi indicating activation of the silkworm systemic immune response (**Fig 5a & 5b**).

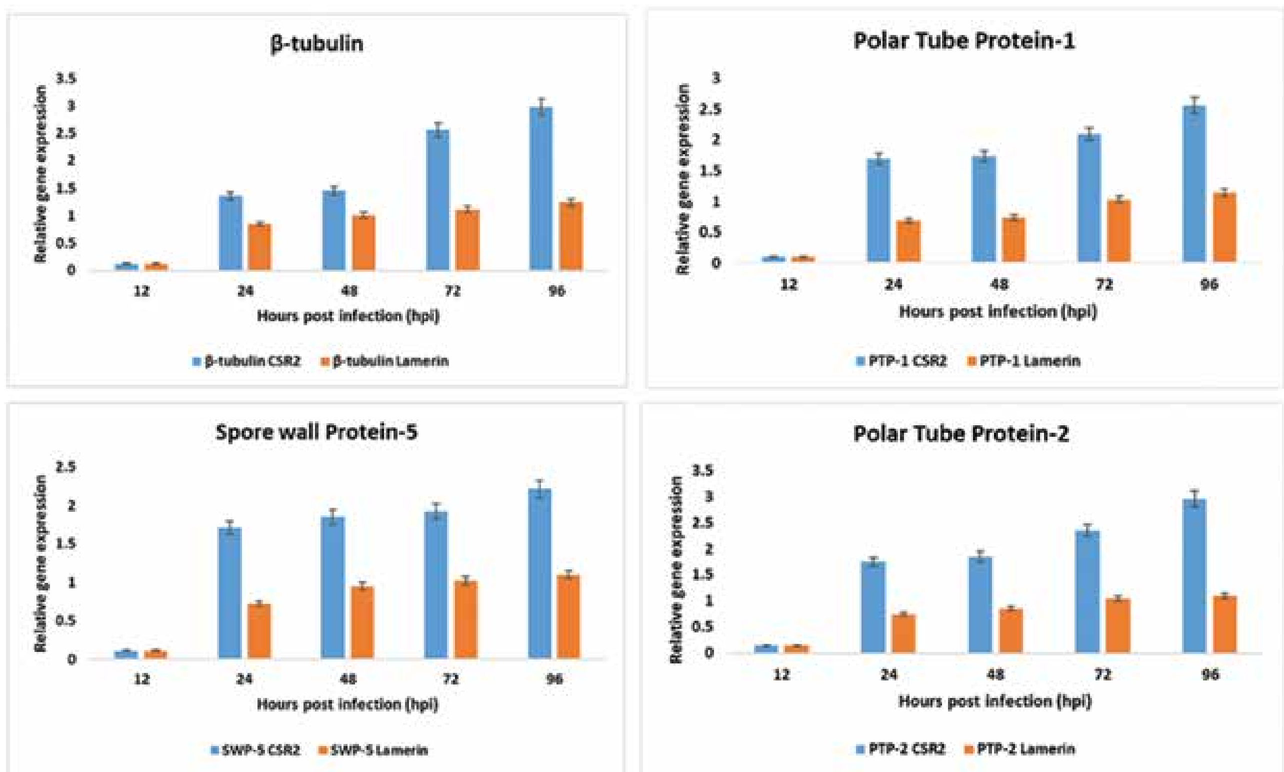
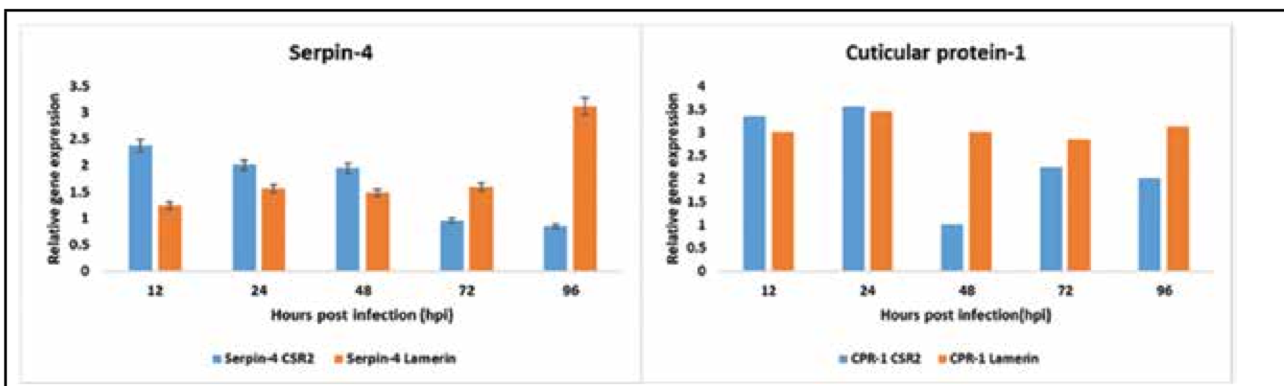



**Figure 5a:** Quantitative PCR analysis *N. bombycis* infected midgut tissue RNA of CSR2 (A) and Lamerin (B) with different *Nosema* specific primers. The RNA of orally infected 3rd instar larvae were collected at different time points starting at 12, 24, 48, 72, 96, hours post infection (hpi).



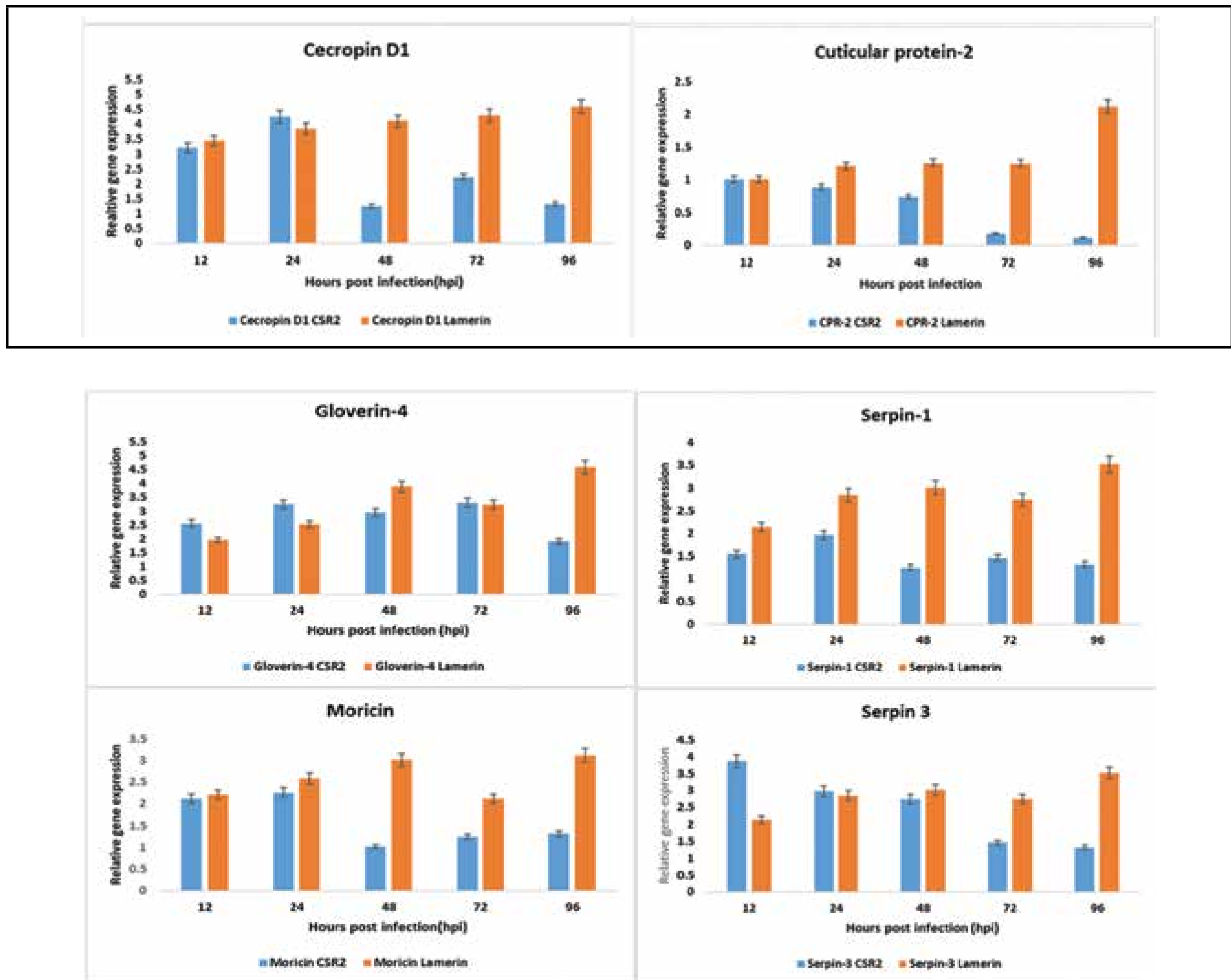



**Figure 5b:**Quantitative PCR analysis *N. bombycis* infected midgut tissue RNA of CSR2 (A) and Lamerin (B) with different *host immune response genes*. The RNA of orally infected 3rd instar larvae were collected at different time points starting at 12, 24, 48, 72, 96, hours post infection (hpi).

## Outcome

Identification of pathogen specific genes that are expressed during infection which are essential for virulence and proliferation in the host is the preliminary information required to prevent and control the diseases. From the studies it was observed that the survivability of Lamerin breeds was > 70% in comparison with < 10% survival rate in the CSR2, the data was supported by the semiquantitative and quantitative PCR results which indicated the presence of pebrine infection in Lamerin and simultaneously the 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 the defense mechanism of CSR2 significantly increased at 72hpi, 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.

## 2. **PIT08004MI: Study on Epigenetic and autophagy modifiers on induction of haploid microspore embryogenesis in mulberry**

(In collaboration with CSGRC, Hosur; **Duration:** March 2020- February 2023)

A. Ramesha, Himanshu Dubey, Raju Mondal* and Prashanth Sangannavar#
*CSGRC, Hosur, #CSB, Bangalore

### Objective:

**1.** To develop a protocol for haploid microspore embryogenesis in mulberry.

To increase the quality and quantity of mulberry leaf production through breeding, understanding genetics of traits of interest are prerequisite. Homozygous/inbred lines are used for trait heritability studies and trait discovery applications such as mapping, gene functional analysis and for development of superior hybrids from the parents lacking undesirable alleles. Successful deployment of doubled haploids through production of haploids through androgenesis and/or gynogenesis by tissue culture methods in many crop plants offers an attractive alternative approach for development of inbred lines in mulberry also.

Basic studies from model plants identified main limiting factors for initiation of microspore embryogenesis are low cellular reprogramming efficiency and high microspores/pollen cell death during stress treatment. In agreement, recent reports on treatments of microspores with epigenetic modifiers, autophagy and proteases were shown to be very promising in terms of enhancing induction of microspore embryogenesis and production of doubled haploids in different crops. Therefore, this project aims to study the effect of chemicals to modify epigenetic status and inhibit cell death in mulberry as reported in few crop plants to enhance haploid microspore embryogenesis and regeneration of haploid plants.

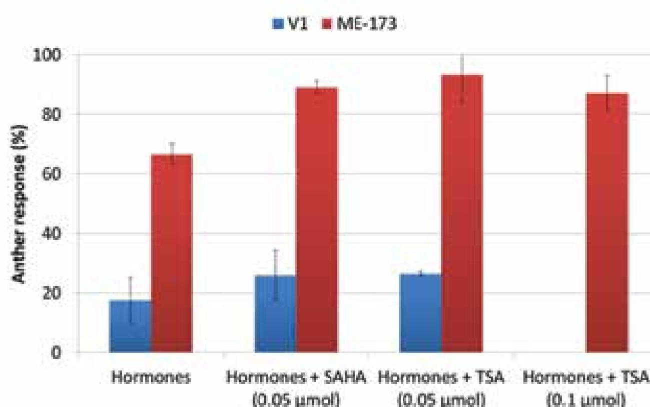



**Figure 6**: Effect of epigenetic modifiers on anther response (%) in V1 and ME-0173 genotypes

The effect of epigenetic modifiers, such as Trichostatin A and SAHA were tested for anther response in V1 and ME-0173 genotypes. The buds were collected and given cold stress at 4°C for 3 days and then anthers were dissected after surface sterilization. The anthers were cultured in different combinations of harmones with or without epigenetic modifiers. Significant improvement of anther response upon treatment of trichostatin A and SAHA were observed in comparision to treatment without epigenetic modifiers in ME-0173 genotype (**Fig. 6**).

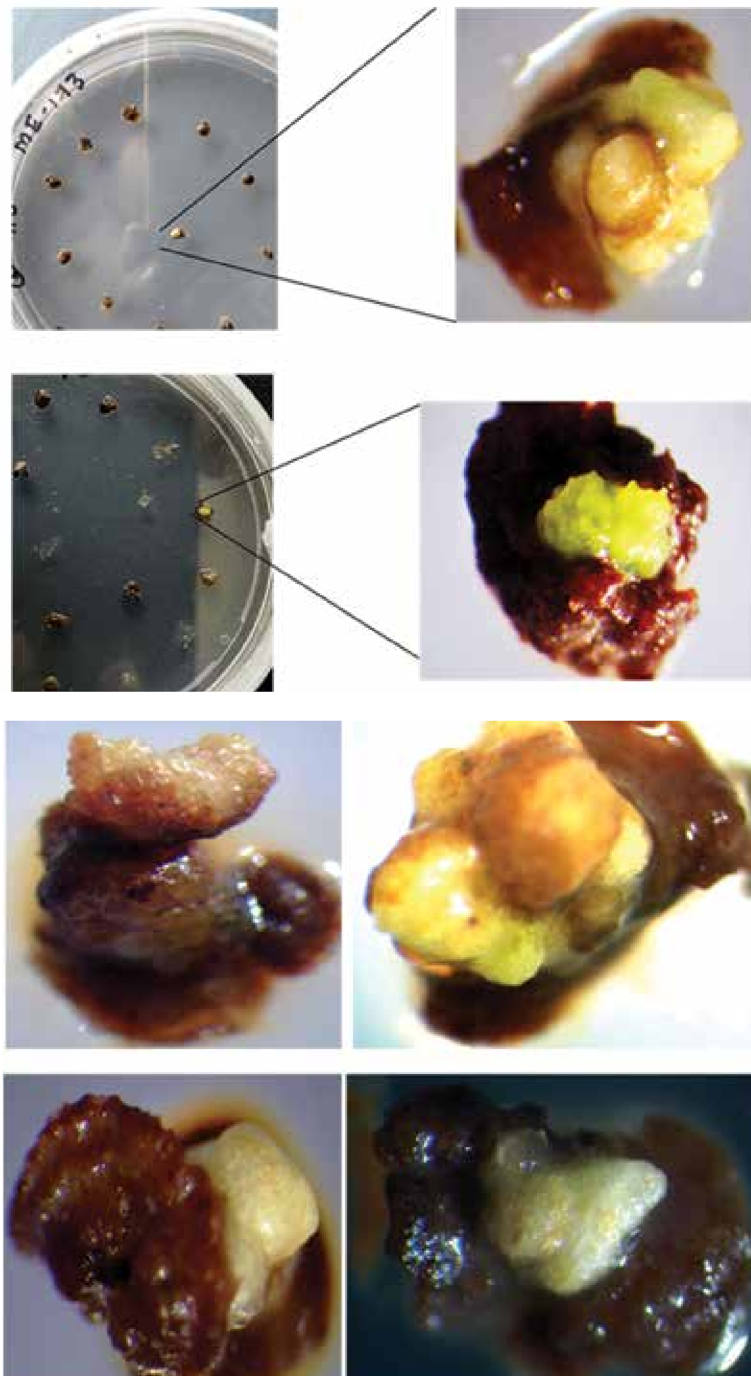



**Figure 7**: Anther response with respect to callus/embryogenic structures in ME-0173 genotypes

However no siginificant differences were noted with treatment of epigentetic modifers in V1 variety. In ME-0173 genotype, the anther response include intially increase of anther size, formation of embryogenc callus and embryo like structures were observed (**Fig. 7**). The developed callus and embryo like structures were transferred to regeneration media containing 0.5 mg/l TDZ in the presence of light.

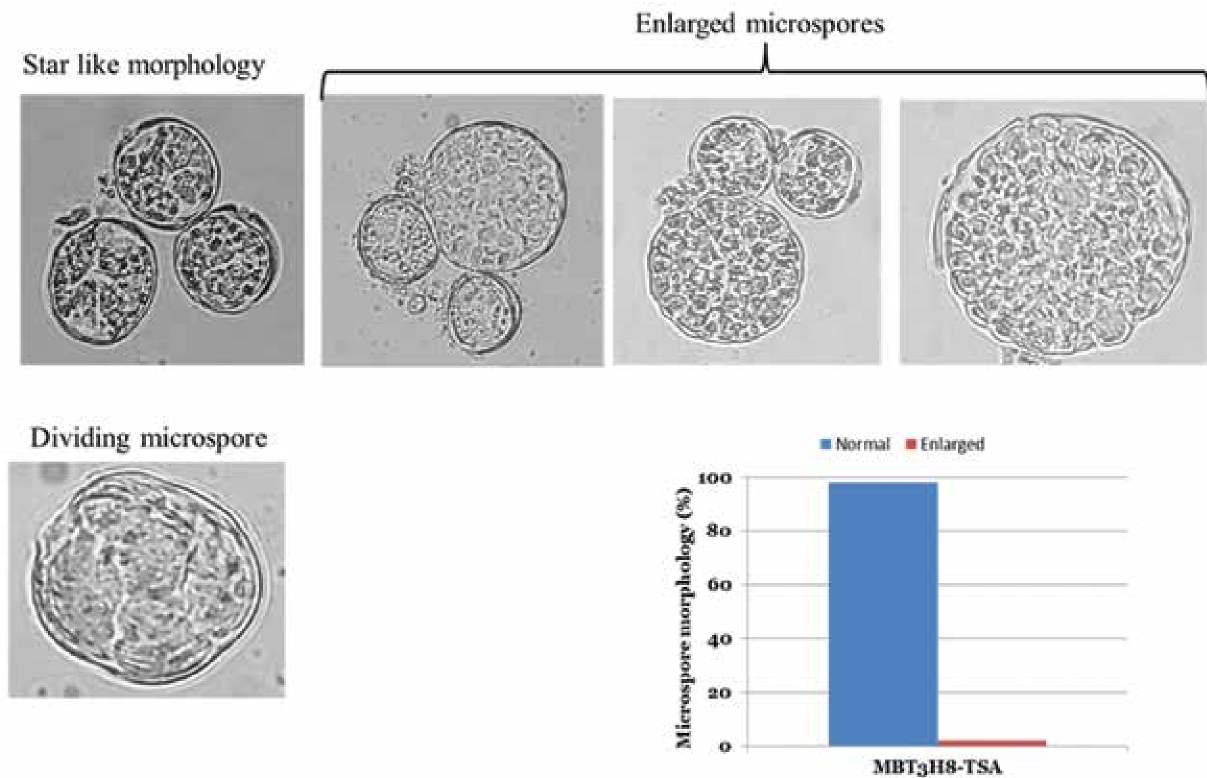



**Figure 8**: Microspores showing star like morphology and enlarged in size after treating with trichostatin A in V1 variety.

The effect of epigenetic modifiers was tested with isolated microspores from V1 and ME-0173 genotypes. The isolated microspores were treated with Trichostatin A at 0.5 and 1 μM and SAHA at 10 μM for 24h and then treated microspores were washed thoroughly and cultured in different combinations and concentrations of hormones such as 2,4-D, kinetin and 6-BAP at 0.5-2 mg/l separately and in combination of 2,4-D with kinetin or 6-BAP. The results showed that most of the microspores do not enlarge and further undergo division. However, very small proportion of microspores observed to be enlarged in combination of 2,4-D and 6-BAP at 0.2mg/l each (**Fig. 8**)

## 4. AIT08005MI: Development and Evaluation of Bidensovirus resistant silkworm hybrids developed from marker assisted breeding lines - Phase II

(Collaboration with CSR&TI, Mysore & CSR&TI, Berhampore; **Duration:** March 2020-February 2023)

K.S. Tulsi Naik, A. Ramesha; M. S. Ranjini* & M. N Chandrashekar*

K. Rahul# & Mihir Rabha#

*CSR&TI, Mysore; #CSR&TI, Berhampore

### Objectives

1. To develop bivoltine silkworm hybrids among marker assisted breeding lines and evaluate for *Bm*BDV resistance.
2. To identify suitable multivoltine/bivoltine parents in the pipeline carrying *Bm*BDV resistance and develop and evaluate cross breed hybrids for *Bm*BDV resistance.

Sericulture has been one of the main branches of agriculture in Asiatic countries since hundreds of years. Like any other lepidopterans, the silkworms also are affected by pathogens especially viruses. Bombyx mori nucleopolyhedrovirus (BmNPV) and Bombyx mori densovirus (BmDNV) now named as a Bombyx mori Bidensovirus (BmBDV) are the major prevalent viruses. Under favorable conditions, Bombyx mori bidensovirus (BmBDV)/ Bombyx mori Densonucleosis virus-2 (BmDNV-2)/BmBDV cause flacherie disease which can bring down cocoon productivity upto 20%. The *nsd-2* gene (non-susceptibility to densovirus-2), which happens to be/which is a putative BmBDV receptor identified to be involved in resistance under recessive mutation condition.The *nsd-2* gene encodes a putative amino acid transporter that was originally predicted to be localized to the membrane & function as a receptor for the entry of DNV-2 virus into the silkworm.The silkworm larvae get infected with BmBDV upon feeding on contaminated mulberry leaves and upon entry into the host, the virus specifically infects and multiplies in the nucleus of midgut columnar cells.The natural deletion occurring in the *nsd-2* gene resulting disruption of gene function has contributed to the evolution of BmDNV-2 resistant silkworm breeds.

### Screening of Berhampore breeds for the presence of resistant and susceptible *nsd-2* gene either in the homozygyous or heterozygous condition.

The moths of 20 bivoltine breeds and 14 multivoltine breeds from CSRTI, Berhampore (**Table 1**) were collected and checcked for the presence or absence of the BmBDV resistant *nsd-2* gene using gene specific primers. From each breed 50 moths were taken and analysed for the presence of the resistant *nsd-2* gene by using the AA1 (for Resistant) and AA3 (for Susceptible) primers.

Out of total 20 bivoltine breeds and 09 multivoltine breeds that were screened for the presence of *nsd-2* resistant marker, the 10 bivoltine breeds NFC18, NFC12, NFC 6, NFC 2, NFC 11, NFC19, BHP3, BHP2, NFC 15, BHP FC1 and all the 08 multivoltine breeds NP1, N Debra, N chalsa, 12Y (CR) , N mark, Mcon4, Nplain, M12W, showed amplification with only *nsd-2* susceptible marker (susceptible to BmBDV) and no amplifications were found for *nsd-2* resistant marker (BmBDV resistant) therefore these breeds are considered to be homozygous susceptible as there is the absence of resistant allele ,whereas the races BHP8, BHP9, NFC8, NFCR, B.con1, Bcon4, BHP FC2, SK6*SK7, SK6, SK7 and 01 multivoltine breed 12Y showed amplifications with both AA1 *nsd-2* resistant marker (resistant to BmBDV) and AA3 *nsd-2* susceptible marker (susceptible to BmBDV) gene specific primers and therefore these races posses the resistant allele either in homozygous/heterozygous conditions. Further these larvae were reared till cocoon formation, and respective male and female moths of particular DFLs (disease free layings) were screened for the presence of resistant allele and check the presence of marker in each DFLs in either homozygous resistance /heterozygous condition (**Fig. 9**).

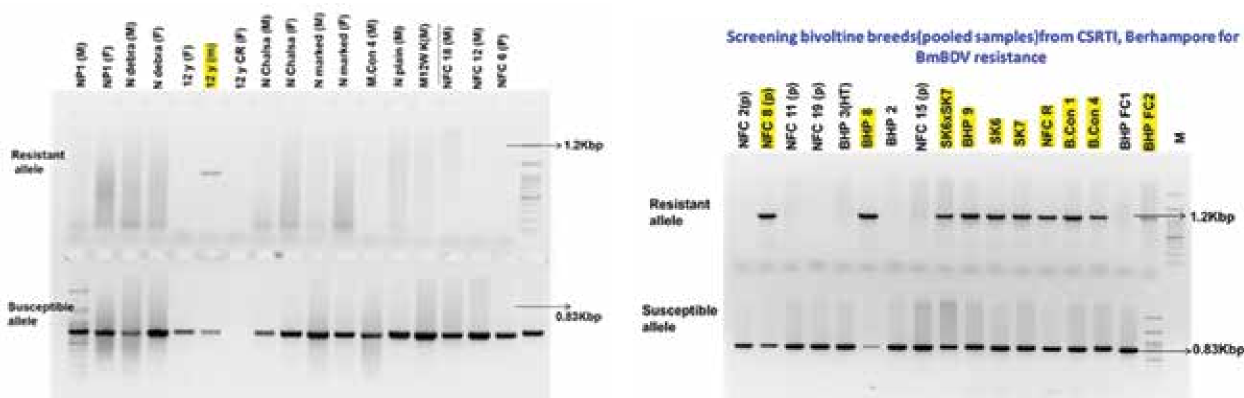



**Figure 9:** Screening of silkworm for the presence of BmBDV marker. The races that showed the presence of BmBDV resistant marker and positive for BmBDV susceptible marker are shown in the same figure.

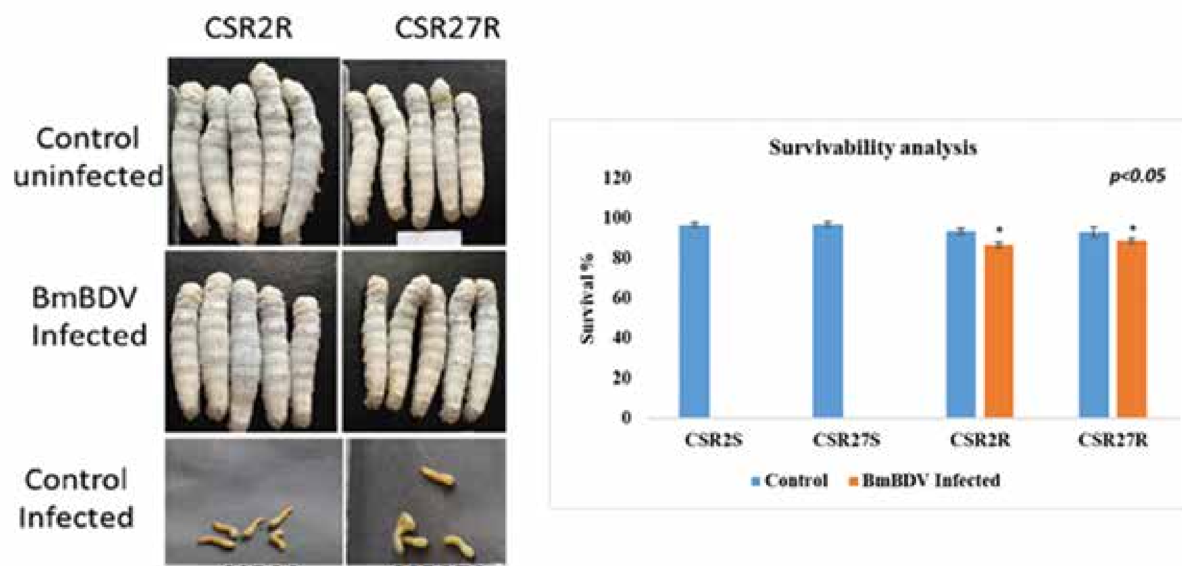
## Table 1: Silkworm bivoltine and multivoltine races screened for the presence of BmBDV resistant marker

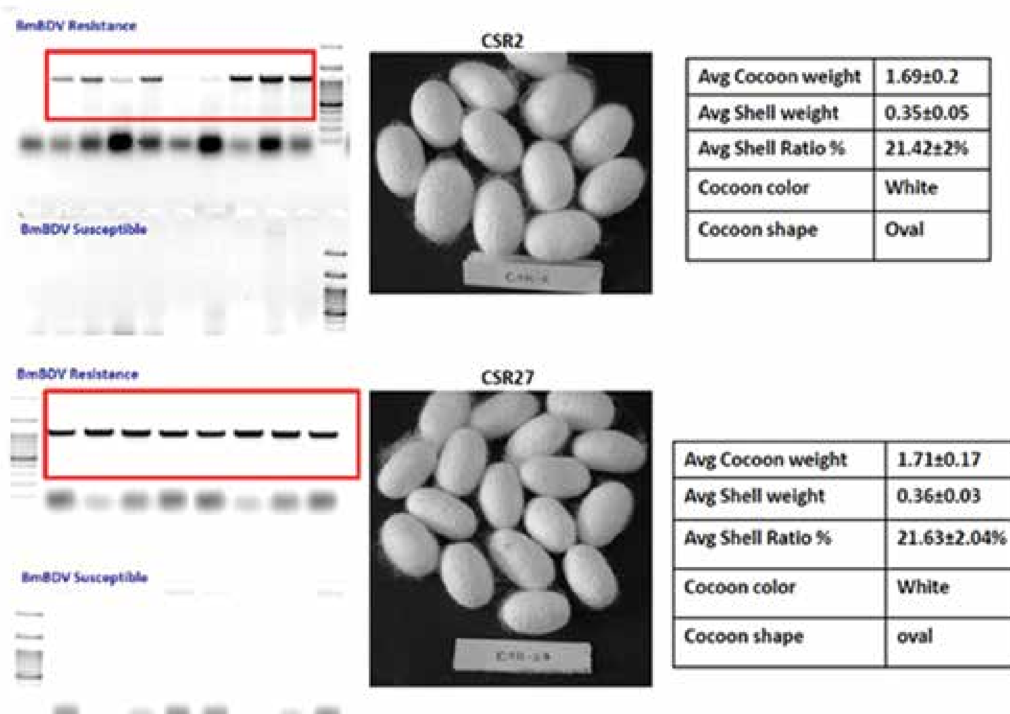
| Sl. No | Silkworm breed (Bivoltine) | Presence/ absence of nsd-2 resistant allele |
|---|---|---|
| 1 | NFC 18 (M) | Absent |
| 2 | NFC 12(M) | Absent |
| 3 | NFC 6 (P) | Absent |
| 4 | NFC 2(P) | Absent |
| 5 | NFC 8(P) | **Present** |
| 6 | NFC 11(P) | Absent |

| 7 | NFC 19 | Absent |
|---|---|---|
| 8 | BHP 3 | Absent |
| 9 | BHP8 | **Present** |
| 10 | BHP2 | Absent |
| 11 | NFC 15 (P) | Absent |
| 12 | SK6xSK7 | **Present** |
| 13 | BHP9 | **Present** |
| 14 | SK6 | **Present** |
| 15 | SK7 | **Present** |
| 16 | NFC R | **Present** |
| 17 | B.Con 1 | **Present** |
| 18 | B.Con 4 | **Present** |
| 19 | BHP FC1 | Absent |
| 20 | BHP FC2 | **Present** |
| **Silkworm breeds (Multivoltine)** | | |
| 1 | NP1 | Absent |
| 2 | N debra | Absent |
| 3 | 12 (Y) | **Present** |
| 4 | 12Y(CR) | Absent |
| 5 | N chalsa | Absent |
| 6 | N marked | Absent |
| 7 | N plain | Absent |
| 8 | M.Con 4 | Absent |
| 9 | M12 W | Absent |

## Transfer of resistance gene to CSR2 and CSR27 and to susceptible parents of commercial hybrids

The CSR2 and CSR 27, productive breeds as well as parental breeds of FC2 are completely susceptible to DNV2 infection as they posses *nsd-2* alleles in homozygous condition therefore to make both the breeds resistant to DNV2 a donar parent is identified. Therefore, both CSR2 and CSR 27 were crossed with J2P BmBDV resistant donar parent and subsequent back crossing with respective recurrent parents was done till BC6 generation generation. The back cross population were screened each generation for the presence of *nsd-2* resistant allele. After 6 generations, the respective CSR2 and CSR27 populations were sibmated to identify homozygous *nsd-2* resistant lines. The sibmated populations were subjected to bioassay to check the survivability and the survived moths were subjected to genotyping to check and confirm the presence of *nsd-2* resistance marker.




| Avg Cocoon weight | 1.69±0.2 |
|---|---|
| Avg Shell weight | 0.35±0.05 |
| Avg Shell Ratio % | 21.42±2% |
| Cocoon color | White |
| Cocoon shape | Oval |

| Avg Cocoon weight | 1.71±0.17 |
|---|---|
| Avg Shell weight | 0.36±0.03 |
| Avg Shell Ratio % | 21.63±2.04% |
| Cocoon color | White |
| Cocoon shape | oval |

**Figure 10**: **A**. Bioassay for homozygous BmBDV resistant CSR 2R, CSR 27R, breeds BmBDV and survival percentage; **B**. Screening individual male and female moths (CSR2 moths) and (CSR27 moths) for BmBDV resistance.

Further, the BmDNV-2 resistant productive breeds like CSR2R and CSR 27R (FC1xFC2 parents), were validated for resistance to BmDNV-2 through bioassays and confirmed the absence of viral genome at molecular level, thereby demonstrating complete resistance of developed breeds upon BmDNV-2 infection.

## 5. **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)

K. M. Ponnuvel, Himanshu Dubey, G Subrahmanyam[1], Mr. Mohammed Muzeruddin Baig[2], & Sailaja Bandam[3]

[1]CMERTI, Ladhoigarh; [2]CTRTI, Ranchi; [3]SSTL, Kodathi

### 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.

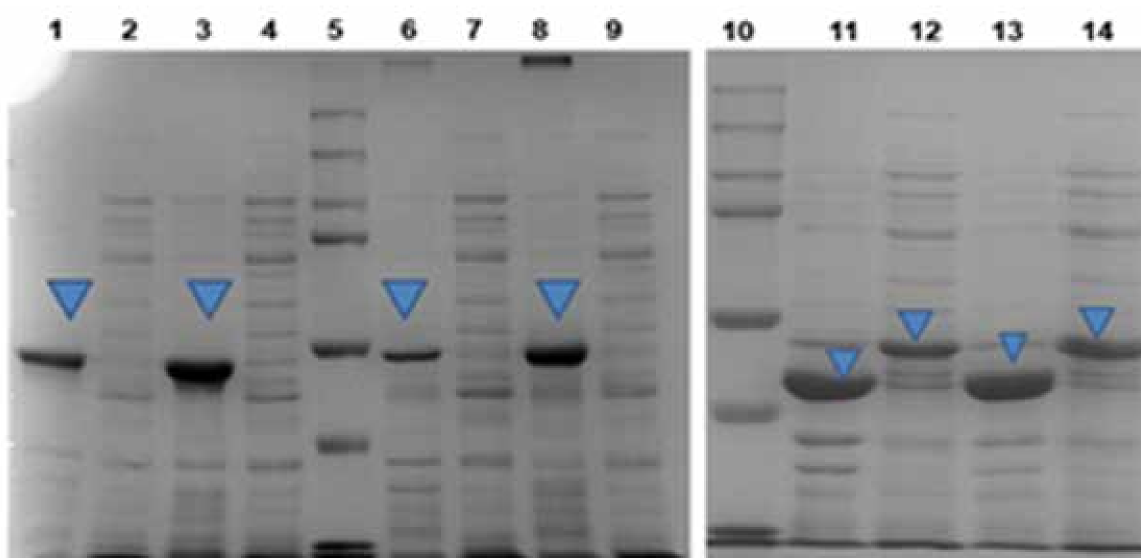



**Figure 11**: SDS page of the expressed proteins of SWP1 and 3. Lane 1- SWP1 in BL21DE3PlysS Pellet, Lane 2- SWP1 in BL21DE3PlysS Supernatant, Lane 3- SWP1 in RosettaPlysS Pellet, Lane 4- SWP1 in RosettaPlysS Supernatant, Lane 5- Marker, Lane 6- SWP3 in BL21DE3PlysS Pellet, Lane 7- SWP3 in BL21DE3PlysS Supernatant, Lane 8- SWP3 in RosettaPlysS Pellet, Lane 9- SWP3 in RosettaPlysS Supernatant, Lane 10- Marker, Lane 11- SWP5 in BL21DE3PlysS Pellet, Lane 12- SWP5 in BL21DE3PlysS Supernatant, Lane 13- SWP5 in RosettaPlysS Pellet, Lane 14- SWP5 in RosettaPlysS Supernatant

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. The potential gene candidates for protein expression and purification were selected as SWP (Spore Wall Proteins) 1 and 3 of *Nosema assamensis* and SWP5 of *N. bombycis* which are found to be localised on the endospore and exospore regions of the microsporidia. These spore wall proteins were identified to be suitable molecular markers to detect microsporidian infection in silkworms. The targeted proteins were cloned and expressed in the *Escherichia coli* expression systems through IPTG induction (**Fig. 11**).

The proteins were expressed in bulk and were subjected to purification and tested on the gel (**Fig. 12**). The molecular size of SWP1, SWP3 and SWP5 were found to be 46 kDa, 52kDa and 35 kDa respectively. These purified proteins have been injected into mice to generate antibodies. The titre values have been evaluated through ELISA and booster doses are being provided for all the three proteins to increase the titre of the antibodies.

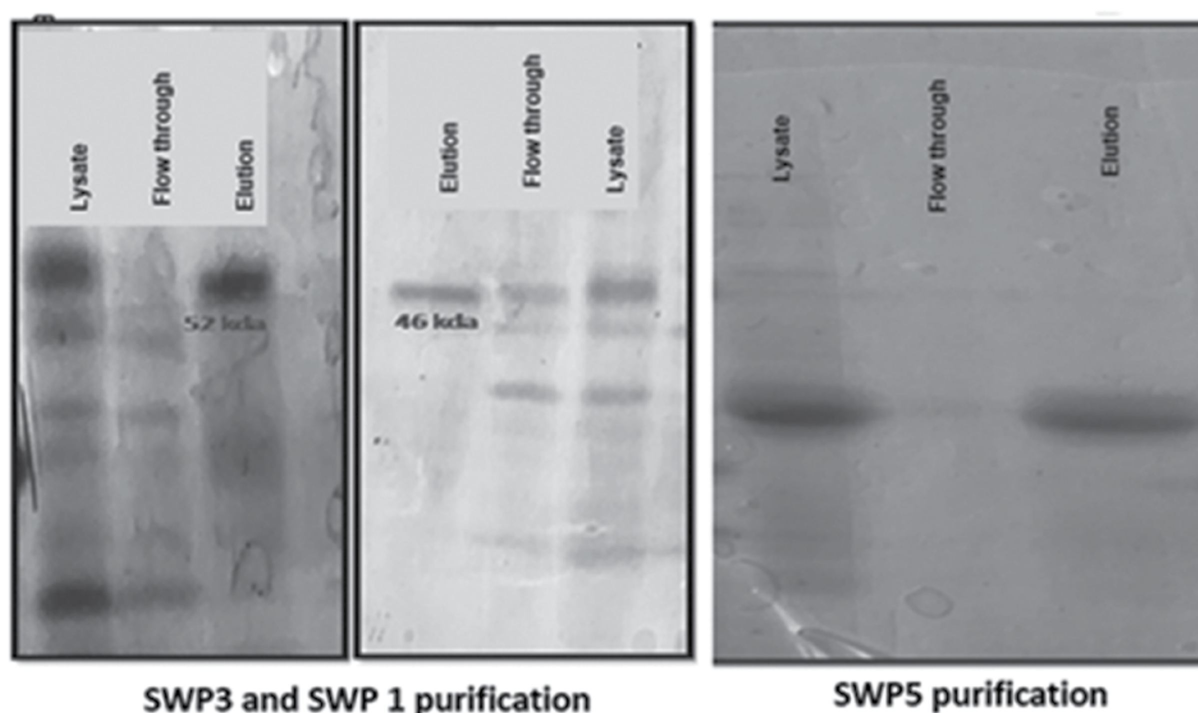



**Figure 12**: Purification of recombinant spore wall proteins 3, 1 and 5 respectively through affinity chromatography

The generated antibodies will be used in western blot analysis and further development of the assay kit. The antibodies against the spore wall proteins will be checked by western blotting before proceeding with the LFA kits.

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

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

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

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

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

### Objectives:

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

Virosis is a dreadful disease in Muga silkworm and an average of 40% crop loss normally occurred due to incidence of disease. The methods attempted to control virosis disease in Muga culture have been found to yield very limited success. This could be due to non-availability of information on viral pathogen, epidemiology and relevant procedures/protocols/diagnostic tools for the early detection of pathogen in the rearing fields and seed production centers. The mortality due to this viral infection predominantly occurs in the late instars and till date no effective control measures have been successfully employed. In this backdrop, 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 will be investigated so as to develop an appropriate molecular based diagnostic technique for early detection of the virus. The project was implemented in March 2022. In the current study, virosis infected Muga samples were collected (**Fig. 13**). Viral pathogen was purified. Viral nucleic acid has been extracted and is found to be segmented RNA. Selective viral genes were amplified, cloned and sequenced. The preliminary PCR results indicate that this virus is a cypovirus type 4, AaCPV-4.

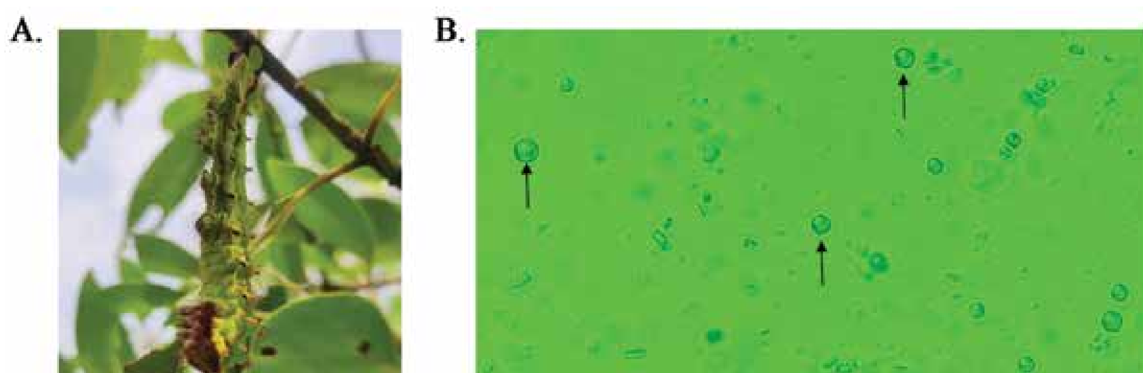



**Figure 13**: **A.** Virosis infected Muga silkworm, *Antheraea assamensis* Helfer; **B**. Light microscopic image of viral polyhedral bodies.

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

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

M. Moorthy[1], R. Debnath, Ramesha A, Himanshu Dubey, Malashree N[2], Reeta Luikham[3], L. Natarajan[4] & P. Kumar[5]

[1]CSB, Bangalore, [2]SSTL, [3]CMER&TI, [4]P2-BSF-Topatoli, [5]ESSO-Hosur

### 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

Improvement in eri raw silk production is required for sustainable ericulture. To achieve this, systematic breeding programmes are required to develop and augment our portfolio of breeds/lines that can be disseminated to farmers according to their needs from time to time. Eri silk has shown potential by drawing significant attention from stakeholders. Thus, improvement in the production and productivity of Eri silk is one of the major mandates of socio-economic development of marginal farmers practicing Eri culture. This newly initiated project tries to support this by developing eri breeds with improved silk/ pupal protein trait by utilizing the genetic variability existing in silkworm strains/morphotypes.

Genetic heterogeneity in eri silkworm and differing geographies have evolved distinct ecoraces of eri silkworm in nature. These ecoraces display attributes in terms of silk color, cocoon properties, larval color/markings and have been reared by farmers in tribal and non-tribal belts in north east. Six strains are known to be prevalent in the eri population categorized according to the larval markings/coloration. Two strains devoid of any markings are classified as yellow plain (YP) and greenish blue plain (GBP) based on integument colour; the rest four are variations of these two body color types based on the pattern of melanization – yellowish zebra (YZ), yellowish spotted (YS), greenish blue zebra (GBZ) and greenish blue spotted (GBP). Earlier researchers were successful in isolating non-segregating lines of YP, YS and GBP indicating 100% homozygosity is possible in few morphotytpes/ strains. In view of this, it is ascertained that lines homozygous to one body colouration and pattern would be useful in developing molecular markers and be utilized in breeding program targeting silk/ pupal protein production

## 8. ARP-08001CI: Studies on the genetic characterization, transmission and tissue distribution of Iflavirus infecting the Indian tropical tasar silkworm, *Antheraea mylitta*

(Funded by Swedish Research council collaboration;

**Duration:** April 2018 – September 2022)

K. M. Ponnuvel, Olle Terenius*, Siripuk Suraporn**, Wenli Li#, Joachim de Miranda* and Helena Bylund*

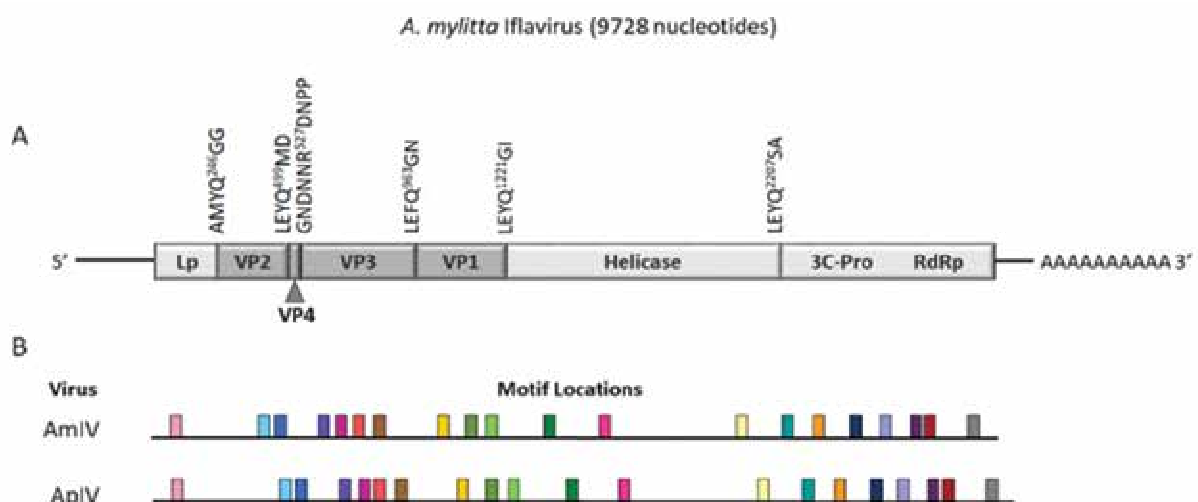
*Swedish University of Agricultural Sciences, Sweden **Maha Sarakham University, Thailand

#Dalian University of Technology, China

### Objectives:

1. To characterize the Iflavirus infecting the two silkworm species, *Antheraea mylitta* and *Antheraea proylei*
2. To analyze the source of infection, tissue tropism, cross-infectivity, biogeographic surveys and life histories
3. To study the effect of Iflavirus infection on the susceptibility status of host silkworms & its impact on infection of other potential pathogens i.e. microsporidian & baculovirus
4. To develop simple & easy diagnostic method for early detection of viral pathogen

The study concluded achieveing all the objectives represented under the this study. As per this, the study identified the causal organism of virosis in *A. mylitta* silkworms to be Iflavirus (called *Antheraea mylitta* Iflavirus- AmIV), the genome sequence deciphered under the accession no. *MW115117.2* on NCBI. The genomic details revealed the typical Iflavirus structure such as genome size of 9728ntds, structural proteins followed by non-structural proteins starting from 5' end towards 3' end. The genome also displays a poly A tail at the 3' end along with the UTR regions on either sides of the genome. The conserved motifs were also mapped between AmIV and *Antheraea pernyi* Iflavirus genome (**Fig. 14**).



| Motif | Symbol | Motif Consensus |
|---|---|---|
| 1. | | EYDGKYEHRHMFGLSSTLDDDRLEDEWRFKYNKLIKHRNWLWFMCKNCKY |
| 2. | | TCDQPNTIPFRIHRYWRGDMVVKIHINCNKFQIGQLQCSWYYQPKADAAF |
| 3. | | YTRSGTHHCVISAAPNNEVELRIPYKAYKSMYHTKTFTGDEKDLPLDLGT |
| 4. | | HNLRLSGRAQTKHPDVDLDEMRIDVLKRKYMLCDVFSWSQQNMNGNVLWS |
| 5. | | AQPGTNLLRSYQLTPIGFLSSLFQYWRGSIEYRFDIVASQFHSGKLLLAY |
| 6. | | PGIAEGESVTIDQARASPHIVISLDNAMSYTWRIPYVADRPWWPRRYAGE |
| 7. | | YMRGGVDMEFAIPVQPAIGLGYDRNYVSSRNTSDVFPVSTTTTFYVGKWH |
| 8. | | HGMLTYGEKFQDLKDLGRRYQIYGWTTVPRTQIERDPGACSFLFPVLPQG |
| 9. | | WVQHRPDRRLERDVITSCTQVTTAEAVFNHTYGVYMQALRVNNVIEIEVP |
| 10. | | QDWSRFYSLGELSVGFFGEQPTADVRCTIYYSMADDCRFTTYQGVPPMV |
| 11. | | AITGFLSLICGGLCTLFGMKNSIKYQPASDCLFKEITNGMRMSNVCFMFF |
| 12. | | CKDQGIVSDTHLMCVLNATSKFWDNCDRQPCLVMDDAFNIKKGPMFEDQ |
| 13. | | ESWMCGMDDRHLVFDKLNFDNIMSKLTKWVWDFYYQQMKPAIKSVFTF |
| 14. | | MSAIDVVYTNADGKDVRVRNYGLMLRDQQMLIQKHYYDFWRRLDVTAKFY |
| 15. | | EHSYIKFDECYLYSSLTDMCMHCVMNVEMNKDVTDSNGWLQLSECYSYKY |
| 16. | | HHQGSVSLYIPSLIHGAYEVDTEPNPLSPSDKRLPPGNPPLKRGVEHMGR |
| 17. | | FEPLEWNSSEGFPLKSLRPPRVKGKKWLFDLEETSNGYVLKGMHGELKRQ |
| 18. | | PVQYTIAFKQYFGDFMASYQEARLDAEHGIGINVDSLEWTQVANYITYYG |
| 19. | | KNFGPSLMLKCVEKAFDIIMKWYERYDNDPERQLIRRVLLSEILHAQHLC |
| 20. | | HPNSKCIFLAPIETQSIRKCVNWITRKGDPLANTLENCKQACELAFGHGP |

**Figure 14: A.** Genome organisation of *A. mylitta* Iflavirus. **B.** Comparison of conserved motifs between AmIV and ApIV (*A. pernyi* Iflavirus)

The conserved motifs look similar to that of Iflavirus. The phylogenetic studies indicated that the identified virus belonged to or closely associated with the Iflavirus derived from *A. pernyi* (showing 91% homology of the amino acid sequence), indicating that these viruses to be similar. The study also explored the conserved motifs in the amino acid sequence of the Iflavirus capsid proteins (**Fig. 15)**. The analysis was performed along with other Iflaviruses. The conserved amino acid residue is highlighted in the figure. The conserved amino acids were also mapped for the helicase and RdRp genes of the Iflavirus.

```
VP2                                                           NxNxFQxG

AmIV   :339 KTTCD------QPNTIPFRIHRYWRGD-MVVKIHINCNKFQIGQLQC
ApIV   :416 VSTCN------QPNTIPFRIHRYWRGD-MIVKIHVNCNKFQIGQLQC
DWV    :311 ---CD------VPNTIPFKVHAYWRGD-MEVRVQINSNKFQVGQLQA
DWV    :311 ---CD------VPNTIPFKVHAYWRGD-MEVRVQINSNKFQVGQLQA
NLHV-3:388 ADSKD------LPNIIPFKISSFSHFDSMEIKFLLNSNKFQTGCLGA
GNV    :240 NSRTDG----SMPILVPFKIYQYFKSD-IHIKFRINSNKFQAGQL--
SBPV   :272 PRYTN------IPNFIPFNIHQYMRAD-FEVKIYVNPNDFVSGWLIM
BrBV   :375 TEFVN------TPVFIPFRNNRLWRGD-LEFKFVLNSNKFQQGSLQV
NLHV-1:348 MKLKS------NPVCVPFLVHNYWKGD-LQFRIVVNANKFQAGQIQA
IsIV   :482 RPFAD------SPNFLMLKKWTYWHFD-LDFRIQVNANRFQVGQLLV
DcPV   :360 SQNYS------ALNSQLFFSHRLFHTN-CKFRLVMNSNRYQMGSLIV
                                                 * *     *
```

```
VP3                                          WxGxLxxxFxFxxxxxxxGxxxxxYxP

AmIV   :626 GTNLLRSYQLTPIGFLSSLFQYWRGSIEYRFDIVASQFHSGKLLLAYIP
ApIV   :703 DTNVLRSYQITPIGFLSSLFQYWRGSIEYRFDIVASQFHSGKLLLAYIP
DWV    :580 GTNPISLYWFAPVGVVSSMFMQWRGSLEYRFDIIASQFHTGRLIVGYVP
DWV    :580 GTNPISLYWFAPVGVVSSMFMQWRGSLEYRFDIIASQFHTGRLIVGYVP
NLHV-3:661 NYEIRRFYRYPPVSVISSCFNLWRGSFIFSLDIVASQFQTCKLICAYIP
GNV    :514 AFVGLDTYAYPPVSVVASLYKQWRGSLEFKFDIISTMFHTGRLLVAYIP
SBPV   :561 TQSKLTQYYLPPISVVSSLYAYTRGSIKYKFLFGNNPRHNARLLVAYIP
BrBV   :647 ANRDYLLYNFPPVTILSNLYAYWRGTLELRLDFVATSFHTGSLLIAYIP
NLHV-1:623 TIESDSCFSIPPVAVASSLFSYWRGCLKMKLDIVGTSMHTGKLLVCYCP
IsIV   :751 TQCHLSIYESTPLAVVSSLFSYWRGSIEFRFDFVASAFHTGRLAFCFVP
DcPV   :649 YRANSGNYVYPPVSVISSCFGYFRGSLKLTIQMIATAFHTGSLMFAIYP
                                        **              *    *

VP1                           FxRG

AmIV   :1051 AREGHIPLIASLFRFYRGSLR
ApIV   :1127 AREGHIPLIASLYRFYRGSLR
DWV    :988  CRDGIIPLIASGYRFYRGDLR
DWV    :988  CRDGIIPLIASGYRFYRGDLR
NLHv-3:1114 CREGPIPLLLSGYRYFRGGLR
GNV    :930  SRDGHIPVISSGYIYFRGSIR
SBPV   :974  MRDGHIPVISSGFRYFRGGLR
BrBV   :1092 CKDGFIPIISSGYRFFRGGLR
NLHV-1:1025 VRDGTIPIVASAYRYVRGSMR
IsIV   :1139 ARAGPMAILANGFRFFRGGLR
DcPV   :1075 AREGLVSLLNTAYLGYTGGLR
               *              *  *

VP4-VP3
                   ▼
AmIV   :553 GNDNNRDNP
APIV   :578 GNDNNRDNP
DWV-A  :466 IGGNNMDNP
DWV-B  :466 VGGCNMDNP
NLHV-3:540 MPDPNRDNP
GNV    :391 -GDTNCDNP
SBPV   :439 -KDVNCDNP
BrBV   :524 DPDANRDNP
NLHV-1:503 LPDHNRDNP
IsIV   :632 LADSNRDNP
DcPV   :525 GRGLNRDNP
                 * * *
```

**Figure 15:** Multiple sequence alignment of capsid protein sequences of Iflavirus. Comparison of conserved amino acids residues between different Iflavirus

The study provided comprehensive details regarding the pathogen, Iflavirus such as the source of infection, tissue tropism and cross infectivity.

The genome structure determination and the presence of capsid proteins has provided a few advantages, where these capsid proteins can be used as potential targets for pathogen identification. To develop a lateral flow assay kit in order to identify the pathogen preliminary work of cloning and expression of the capsid proteins has been achieved. The method holds promises to deliver easy to use point of care kits for the detection of the pathogen.

## SILKWORM STOCK MAINTENANCE

1. Six lines of Transgenic CSR4 and CSR27 breeds with NPV tolerance 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 28th & 29th meeting of the Research Advisory Committee was held on 27th August, 2021 & 4th March, 2022 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 Krishnakumar, Former DDG (Horticulture), ICAR, New Delhi.

### Trainings / Workshops Attended

1. **Dr. Pawan Shukla**, Scientist C attended the Online Hands-on Workshop on "Introductory Genomic and NGS Analysis" Organized by Institute of Bioinformatics and Applied Biotechnology (IBAB) on 22nd -24th April 2021.
2. **Dr. Rajal Debnath**, Scientist C attended the Cloud - based Hands on Workshop on "Molecular Docking, Pharmacophore Modeling and Machine Learning" organised by the Bioinformatics Division, ICMR – National Institute of Cancer Prevention and Research, Noida and Schrodinger Inc. from 15th - 16th March, 2022
3. **Dr. K. S. Tulsi Naik**, Scientist C attended short term training (15 days) on **"Advanced Bioinformatics & NGS- (Reference Based RNA Seq Data Analysis)" at Arraygen Technologies Pvt Ltd during May 2021**
4. **Dr. Pawan Shukla** and **Dr. G. Subrahmaniyam**, attended a Workshop on "Socio Economic Assessment of Sericulture Technology" on 20th to 23rd December, 2021 at CSB, Bengaluru.
5. **Dr. K. Tulsi**, **Dr. Pawan Shukla** and **Dr. Himanshu Dubey** attended Training on GeM portal on 09.12.2021 at CSB, Bengaluru
6. **All the Scientists** of this Institute attended Hands on training on "Application of Statistical Tools in Sericulture Research" from 23th to 24th November, 2021 at CSB, Bengaluru.

### *Abstracts published in Workshops*

1. Pawan Shukla, Ramesha A. Reddy, Kangayam M. Ponnuvel, Gulab Khan Rohela, V. Sivaprasad (2021). Comparative genomics Approach for Identification of Candidate Genes Associated with Low Temperature Stress Tolerance in Mulberry Varieties. Page No.83. International Symposium on Plant Biotechnology Towards Improving Agri-Food Industry and Healthcare Products. Organized by Department of Bioengineering and Biotechnology, Birla Institute of Technology, Mesra, Ranchi, India from 27th-30th October, 2021.
2. K S Tulsi Naik, Sahar Ismail, A R Pradeep, R K Mishra (2021) "Molecular characterization of the functional genes associated with silk assembly, transport and protection in the silk glands of popular multivoltine breeds of silkworm *Bombyx mori*. L" International conference on Biotechnology and biological sciences held at Institute of engineers, Kolkata pg no 73 MEDI-PA-19
3. Ramesha A, Himanshu Dubey, K. Vijayan, Kangayam M. Ponnuvel, V. Sivaprasad, Rakesh K. Mishra, K. Suresh. Genome wide identification of candidate *MLO* genes and genetic variants involved in powdery mildew disease susceptability in mulberry. "International Symposium on Plant Biotechnology Towards Improving Agri-Food Industry and Healthcare Products (ISPB-2021)" October 27, 2021 to October 30, 2021 at Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India.
4. के. एस. तुलसी नायक*[1] सहर इस्माइल[1], शंभवी हुनगगुन्नद, ए. आर. प्रदीप[1], के. एम. पोन्नुवेल[1], राकेश कुमार मिश्र (2021) बहुभिन्नरूपी रेशम नसलों के रेशम के रेशा से जुड़े महत्वपूर्ण जीनों का आणविक आवर्तन Ansh Souvenir pg no: 65-77 CSIR-NAL, Bangalore on 13th Aug. 2021.

## 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। साथही 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.
- मार्च 2021 में SSTL और RSRS, के साथ मिलकर एक दिवसीय संयुक्त कार्यशाला का आयोजन किया गया।
  One joint workshop was organized in March 2021 jointly with SSTL and RSRS, Kodathi.
- हिंदी पखवाड़ा 1 से 14 सितंबर 2021 तक मनाया गया और विभिन्न कार्यक्रम आयोजित किए गए।

# PUBLICATIONS

1. Ponnuvel KM, de Miranda JR, Terenius O, Li W, Ito K, Khajje D, Shamitha G, Jagadish A, Dubey H, Mishra RK. Genetic characterisation of an Iflavirus associated with a vomiting disease in the Indian Tropical tasar silkworm, *Antheraea mylitta*. Virus Res. 2022 Jan 30:198703.
2. Makwana P, Dubey H, Pradeep ANR, Sivaprasad V, Ponnuvel KM, & Mishra RK (2021). Dipteran endoparasitoid infestation actively suppressed host defense components in hemocytes of silkworm *Bombyx mori* for successful parasitism. Animal Gene, 22, 200118.
3. Gupta T, Raghavendar G, Terenius O, Ito K., Mishra RK, & Ponnuvel KM (2022). An investigation into the effects of infection and ORF expression patterns of the Indian bidensovirus isolate (BmBDV) infecting the silkworm *Bombyx mori.* Virus Disease, 1-8.
4. Jagadish A, Dubey H, Kamatchi I, Pradeep AR, Subrahmanyam G, Mishra RK, Ponnuvel KM (2021). Transcriptome analysis of *Nosema assamensis* infecting muga silkworms (*Antheraea assamensis*) reveals insights into candidate pathogenicity related genes and molecular pathways required for pathogenesis. Annals of Parasitology, 67: 671-682.
5. Jagadish A, Khajje D, Tony M, Nilsson A, de Miranda JR, Terenius O, Dubey H, Mishra RK, Ponnuvel KM (2021). Development and optimization of a TaqMan assay for *Nosema bombycis*, causative agent of pébrine disease in *Bombyx mori* silkworm, based on the β-tubulin gene. Journal of Microbiological Methods 186, 106238. https://doi.org/10.1016/j.mimet.2021.106238
6. Kalita M, Chutia M, Jha DK, & Subrahmanyam G (2022). Mechanistic understanding of *Gordonia* sp. in biodesulfurization of organosulfur compounds. Current Microbiology, 79(3), 1-15
7. Goswami J, Gogoi DK, Rasid N, Handique BK, Subrahmanyam G, Bora PP, & Raju PLN (2021). Development of a Muga disease early warning system–a mobile-based service for seri farmers. Current Science, 121(10), 1328-1334.

### Books/Book chapters

1. Gurtler V, & Subrahmanyam G (2021). Methods in Silkworm Microbiology. Vol 49, Elsevier, Academic Press.
2. Subrahmanyam G, Kumar A, Luikham R, Kumar JS, & Yadav AN (2021). Global Scenario of Soil Microbiome Research: Current Trends and Future Prospects. In Soil Microbiomes for Sustainable Agriculture (pp. 573-603). Springer, Cham

# AWARDS

## Paper / poster presentation

1. **Dr. K. S. Tulsi Naik**, Scientist C received the best paper presentation award for the paper entitled "Molecular characterization of the functional genes associated with silk assembly, transport and protection in the silk glands of popular multivoltine breeds of silkworm *Bombyx mori*. L" at the International conference on Biotechnology and biological sciences held at Institute of engineers, Kolkata

## Ph. D. awarded [University of Mysore]

1. **Ms. Dyna Susan Thomas** (Guide: Dr. Ravi Kumar, Scientist D)

   **Thesis Title**: Recombinant Production of Sericin and Sericin-Cecropin B fusion protein and their effects in wound healing and cell culture applications

# RESEARCH ADVISORY COMMITTEE

| SN | Details of Committee | Designation |
|---|---|---|
| 1 | **Dr. N. K. Krishnakumar,**<br>Retired Deputy Director General (Horticulture)<br>Indian Council of Agricultural Research (ICAR)<br>New Delhi | **Chairperson** |
| 2 | **Dr. Sanjay Ghosh**<br>Institute of Bioinformatics and Applied Biotechnology<br>Bangalore | Member |
| 3 | **Dr. Mohan**<br>Principal Scientist<br>Division of Entomology, NBAIR,<br>Bangalore | Member |
| 4 | **Dr. Nataraj Karaba**<br>Professor,<br>Dept. of Crop Physiology, UAS, GKVK,<br>Bangalore | Member |
| 5 | **Dr. K. Vijayan,**<br>Retired Scientist-D,<br>Central Silk Board | Member |
| 6 | **The Director**<br>CSR&TI, Mysore | Member |
| 7 | **The Director**<br>CSGRC, Hosur | Member |
| 8 | **The Director**<br>Seri-Biotech Research Laboratory<br>Kodathi, Carmelram post, Sarjapur Road<br>Bangalore | Member- Convener |

# INSTITUTE BIO SAFETY COMMITTEE

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

# HUMAN RESOURCES

| SN | Name | Designation |
|---|---|---|
| 1 | Dr.V. Sivaprasad | Director |
| 2 | Dr. K. M. Ponnuvel | Scientist- D |
| 3 | Dr. K. S. Tulsi Naik | Scientist- C |
| 4 | Dr A. Ramesha | Scientist- C |
| 5 | Dr. Pawan Shukla | Scientist- C |
| 6 | Dr. G. Subrahmanyam | Scientist- C |
| 7 | Dr. Rajal Debnath | Scientist- C |
| 8 | Dr. Himanshu Dubey | Scientist-B |
| 9 | Mr. R. N. Srikantaiah | Senior Technical Assistant |
| 8 | Mr. K. M. Humayun | Senior Technical Assistant |
| 9 | Mr. G. Raghavendar | Field Assistant |

## Research fellows / Assistants

| SN | Name | Designation |
|---|---|---|
| 1. | Ms. Anupama Jagadish | Ph.D. Scholar & Senior Research Fellow |
| 2 | Ms. Dyna Susan Thomas | Ph.D. Scholar |
| 3 | Ms. Chitra Manoharan | Ph.D. Scholar |
| 4 | Ms. Sandhya Rasalkar | Ph.D. Scholar |
| 5 | Ms. Deeksha Khajje | Ph.D. Scholar & Junior Research Fellow |
| 6 | Ms. Aneesha P.J. | Junior Research Fellow |
| 7 | Ms. Indumathi Kamatchi | Ph.D. Scholar & Junior Research Fellow |
| 8 | Ms. Vanitha C. | Ph.D. Scholar & Project Assistant |
| 9 | Ms. Arathi | Junior Research Fellow |
| 10 | Mr. Naleen | Junior Research Fellow |

## Administration

| SN | Name | Designation |
|---|---|---|
| 1 | Shri R Ranganath | Asst. Director (A & A) |
| 2 | Shri Shaik Ramthu Bhasa | Asst. Director (Official Language) |
| 3 | Ms. Manjula S. | Superintendent |
| 4 | Shri Chandrashekhar Rao | Asst. Superintendent |
| 5 | Shri Kenchappa | Multi tasking Staff |

# SUPERANNUATION

Dr. R. K. Mishra, Director; Shri R Ranganath, Asst. Director (A&A); and Shri Shaik Ramthu Bhasa, Asst. Director (Official Language) retired from service of Central Silk Board (Government of India) on attaining superannuation at the age of 60 years. The fraternity of SBRL thanks them for their esteemed service and wish them happy retired life.

# OTHER ACTIVITIES

## Swachhta Pakhwada

Swachhta Pakhwada was organized from 01st to 15th March 2022 jointly with Silkworm Seed Technology Laboratory (SSTL), Kodathi and RSRS, Kodathi. Various programme were organized as per central office instructions.