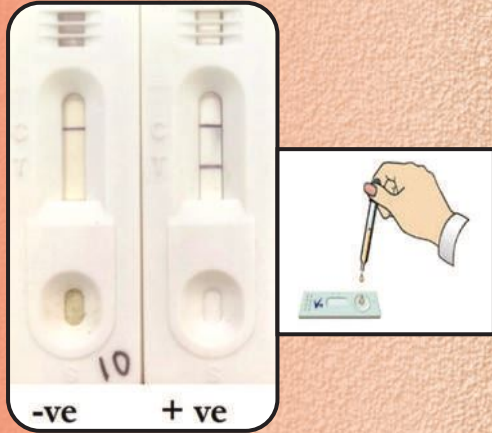


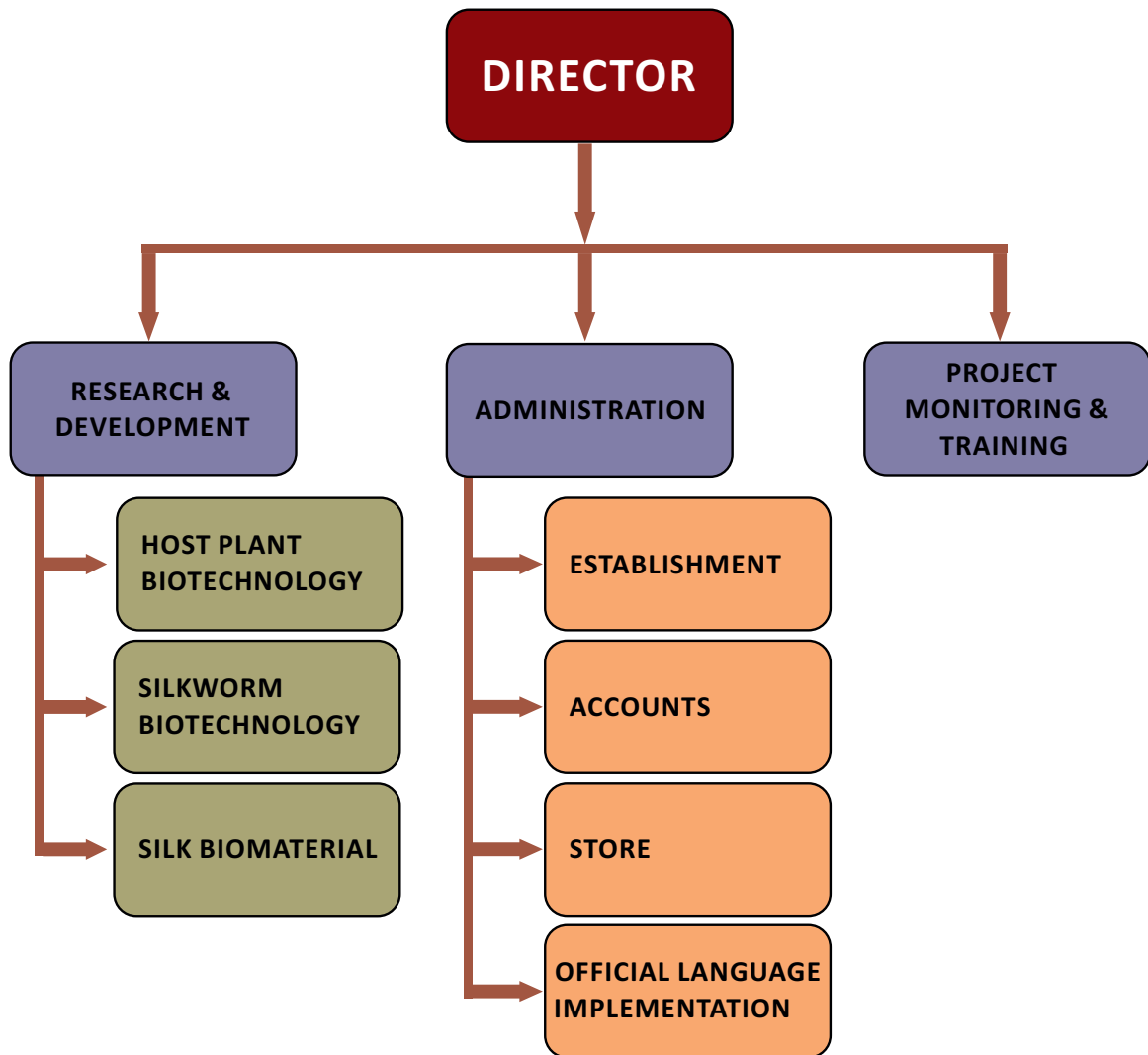
वार्षिक प्रतिवेदन Annual Report 2022-23



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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रेशम जैव-प्रौद्योगिकी अनुसंधान प्रयोगशाला

केन्द्रीय रेशम बोर्ड, वस्त्र मंत्रालय-भारत सरकार

कोड़ती, कार्मेलराम डाक, बेंगलूरु - 560 035

SERI-BIOTECH RESEARCH LABORATORY

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Carmelram post, Kodathi, Bengaluru - 560 035

Published by	:	Dr. S. Manthira Moorthy Director Seri-Biotech Research Laboratory Bengaluru - 560 035
Editorial Committee	:	Dr. Pawan Shukla Dr. G. Subrahmanyam Dr. K. S. Tulsi Naik Dr. A. Ramesha Dr. Rajal Debnath Dr. Himanshu Dubey
Hindi Translation	:	Dr. Pawan Shukla
Cover Page Design	:	Ravindra S. Badiger
No. of Copies	:	50 copies
Printed at	:	M/s. Vishruti Prints No. 53/6, Opp. Bhadrappa Industrial Estate Magadi Main Road, Kamakshipalya Bengaluru - 560 079 Tel: +91 80 40992319

COVER PAGE

Top most image on the cover page depict the Lateral Flow Assay (LFA) kit demonstration and image at the center display the cut opened ovary culture from G4 explants.

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प्राक्कथन



मुझे रेशम जैव-प्रौद्योगिकी अनुसंधान प्रयोगशाला [एस बी आर एल], केन्द्रीय रेशम बोर्ड, कोड़ती कि वर्ष 2022-2023 की वार्षिक रिपोर्ट प्रस्तुत करने में अत्यंत हर्ष महसूस हो रहा है। वैज्ञानिक समझ और नवाचार की खोज में, रेशम के कीड़ों का अध्ययन एक विशेष स्थान रखता है, जो सदियों पुरानी परंपरा को आधुनिक वैज्ञानिक शोध के साथ जोड़ता है। यह रिपोर्ट वन्या और शहतूत दोनों क्षेत्रों में कार्य कर रहे शोधकर्ताओं की हमारी समर्पित टीम के अथक प्रयासों का नतीजा है।

यह वार्षिक रिपोर्ट कई महत्वपूर्ण निष्कर्षों पर प्रकाश डालती है। कुछ प्रमुख उपलब्धियों में शहतूत में चूर्णित आसिता रोग से सम्बंधित संवेदनशीलता के लिए संभावित उम्मीदवार जीन के रूप में दो मिल्डेयु प्रतिरोधी लोकस ओ (एमएलओ) जीन, एम. एल. ओ.-2 और एम. एल. ओ.-6ए की पहचान और लक्षणों का गहनता से अध्ययन किया है। इसके अलावा, अगुणित कैलस/पौधों की जांच के लिए एकल लोकस एस.एस.आर. मार्करों की पहचान की गई और साथ ही बीजांड में अगुणित भ्रूणजनन/कैलस के लिए योगदान देने वाले कारकों की पहचान की गई है। मार्कर असिस्टेड सेलेक्शन का सफलतापूर्वक उपयोग करके बॉम्बिक्स मोरी बाई-डेंसो वायरस प्रतिरोधी संकर रेशमकीट का विकास किया।

इस अवधि के दौरान, संस्थान ने एक लेटरल फ्लो एसे (एल.एफ.ए.) किट का विकास किया है जिसके द्वारा रेशमकीटों में पेब्राइन रोग का पता आसानी से लगा सकते हैं। यह पेब्राइन का पता लगाने की पारंपरिक सूक्ष्म परीक्षण पद्धति की श्रमसाध्य प्रक्रिया को बदलने में मदद करेगा। वर्ष की एक अन्य उपलब्धियों में विशिष्ट रोगाणुरोधी जीन (सर्पिन) की पहचान है, जिसका अपरेगुलेशन माइक्रोस्पोरिडिया संक्रमित लैमेरिन नस्ल में देखा गया, जो नोसेमा बॉम्बिसिस संक्रमण के प्रति सहिष्णुता का संकेत देता है।

अनुसंधान एवं विकास उपक्रमों को सुविधाजनक बनाने में अपने समर्थन और मार्गदर्शन के लिए मैं केन्द्रीय रेशम बोर्ड के सदस्य सचिव एवं निदेशक (वित्त) के प्रति हार्दिक आभार व्यक्त करता हूँ। मैं इस संस्थान की अनुसंधान सलाहकार समिति का भी आभार व्यक्त करना चाहूँगा जिन्होंने अनुसंधान प्रस्तावों के निष्पादन में अपना मार्गदर्शन दिया। साथ ही धन्यवाद देता हूँ, डी.बी.टी. जैसी प्रायोजक एजेंसी का जिन्होंने समय पर अनुसंधान प्रस्तावों के लिए वित्तीय सहायता प्रदान किया। इसी तरह, आई.आई.एस.सी. जैसे सहयोगी भागीदारों के योगदान का भी आभारी हूँ।

मुझे यकीन है कि पाठक इस पुस्तक को अत्यधिक उपयोगी और ज्ञानवर्धक पाएंगे।

डॉ. एस. मंदिरा मूर्ति

निदेशक

FOREWORD

It is with great pleasure and enthusiasm, I present the annual report of Seri-Biotech Research Laboratory [SBRL] Central Silk Board, Kodathi for the year 2022-2023. In the pursuit of scientific understanding and innovation, the study of silkworms holds a special place, intertwining centuries-old tradition with modern scientific inquiry. This report is an outcome of tireless efforts of our dedicated team of researchers working in both vanya and mulberry sectors.



The annual report highlights the many significant findings. Some of the salient achievements includes the identification and characterization of two *Mildew Resistant Locus O* (*MLO*) genes, *MLO2* and *MLO6A* as potential candidate genes for susceptibility to powdery mildew in mulberry. In addition, Single locus SSR markers for screening of haploid callus / plants were identified as well as the factors contributing for haploid embryogenesis/callus in ovule were established. We have also successfully developed BmBDV (*Bombyx mori* Bidenso virus) resistant silkworm hybrids through Marker Assisted Selection.

During this period, Institute has also come up with a LFA kit, entailing implementing of Lateral Flow Assay (LFA) technology to detect pebrine disease in silkworms. It will help in replacing a laborious process of conventional microscopic examination method of pebrine detection. Another achievement of the year is identification of specific antimicrobial genes (serpins) which displayed upregulation in microsporidia infected Lamerin breed indicating tolerance to *Nosema bombycis* infection.

I extend our heartfelt gratitude to the Member Secretary and Director (Finance) of the Central Silk Board for their unwavering support and guidance in facilitating our R&D ventures. My sincere gratitude to the Research Advisory Committee for their guidance in execution of the research proposals. I also thank the sponsoring agency i.e. DBT, GoI for providing financial support to carry out the project. Similarly, the contributions from collaborating partners such as IISc are also gratefully acknowledged.

I am certain that the readers will find this volume useful and informative.

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. एक तीव्र, विश्वसनीय, विशिष्ट और संवेदनशील पेब्राइन का पता लगाने वाली तकनीक, एलएफए (लेटरल फ्लो एसे) किट विकसित की गई।
2. मूगा और तसर रेशम कीट से सम्बंधित 03 अलग-अलग रोगजनकों नोसेमा एसपी., साइपोवायरस-4 और इफ्ला वायरस संक्रमण का एक साथ पता लगाने के लिए एक मल्टीप्लेक्स पीसीआर एसे विकसित किया गया।
3. शहतूत में अगुणित कैलस/पौधों की जांच के लिए एकल लोकस एसएसआर मार्कर विकसित किया गया।
4. शहतूत में चूर्णित आसिता रोग के प्रति संवेदनशीलता के लिए संभावित उम्मीदवार जीन के रूप में दो मिलेड्यु प्रतिरोधी लोकस ओ (एमएलओ) जीन, एमएलओ2 और एमएलओ6ए की पहचान की गई और एमएलओ2 में वैकल्पिक स्प्लिसिंग देखी गई, जिसके परिणाम स्वरूप चूर्णित आसिता रोग के प्रति सहनशील शहतूत में ट्रंकेटेड (खण्डित) प्रोटीन का उत्पादन हुआ।
5. आणविक मार्कर के माध्यम से CSR16, CSR16N, DUN17, DUN18, NFC 8(P), NFCR, BHP8, BHP9, B.con1, B.con4, BHP FC 2, और लैमेरिन में BmBDV प्रतिरोधी एलील की पहचान की गई।
6. मूगा रेशम कीट का संपूर्ण जीनोम सीक्वेंस और ट्रांस्क्रिप्टोम विश्लेषण को पूरा किया गया और इससे उत्पन्न डेटा का उपयोग करके वन्य रेशम कीट के तुलनात्मक जीनोमिक विश्लेषण के लिए वान्या सिल्कबेस सीएमईआर एंड टीआई, जोरहाट के सहयोग से विकसित किया गया।

RESEARCH HIGHLIGHTS

1. Developed a rapid, reliable, specific and sensitive pebrine detecting technology, LFA (Lateral flow assay) kit.
2. A multiplex PCR assay developed for simultaneous detection of 03 different pathogens, *Nosema* sp, cypovirus-4 and iflavivirus infections in both Muga and Tasar silkworms.
3. Developed single locus SSR markers for screening haploid callus / plants in mulberry.
4. Identified two *Mildew Resistant Locus O (MLO)* genes, *MLO2* and *MLO6A* as candidate genes for susceptibility to powdery mildew in mulberry and observed alternative splicing of *MLO2* resulting in generation of truncated protein in powdery mildew tolerant mulberry accessions.
5. BmBDV resistant allele identified in CSR16, CSR16N, DUN 17, DUN 18, NFC 8 (P), NFCR, BHP 8, BHP9, B.con1, B.con 4, BHP FC2, and Lamerin through molecular marker.
6. Whole genome and transcriptome analysis of Muga silkworm was completed. The generated data was utilized for development of Vanya silkbases for comparative genomic analysis of wild silkworms in collaboration with CMER&TI, Jorhat.

LIST OF RESEARCH PROJECTS

ON-GOING 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**
3. **ARP-08007MI** : Biological and molecular characterization of virosis in Muga silkworm, (*Antheraea assamensis* Helfer) (March 2022- February 2025)
4. **AIB-08008MI** : Development and Evaluation of Eri silkworm (*Samia ricini* Donovan) breeds/hybrids with improved productivity (March 2022- February 2025)
5. **AIC-08009CN** : Profiling of lipid, protein and carbohydrate of mulberry mealybug *Maconellicoccus hirsutus* (Green) for its control (April 2022-March 2025)
6. **AIT-08010MI** : Transcriptome studies for susceptibility of Muga silkworm (*Antheraea assamensis* Helfer) to diseases (April 2022- March 2026)
7. **AIE-08011MI** : Evaluation of NPV tolerant bivoltine hybrids and cross breeds at farmer's level (May 2022 - June 2024)

CONCLUDED RESEARCH PROJECTS

1. **PRP-08002MI** : Identification of powdery mildew resistant genes and validation of CAPS marker for Chalcone synthase in mulberry Spp. Caused by *Phyllactinia corylea* (Pers.) Karst. (May 2019- May 2022)
2. **PIT-08004MI** : Study on epigenetic and autophagy modifiers on induction of haploid microspore embryogenesis in mulberry (March 2020- March 2023)
3. **AIT-08005MI** : Development and evaluation of Bidensovirus resistant silkworm hybrids developed from marker assisted breeding lines -Phase II (March 2020- February 2023)

PROGRESS OF ONGOING RESEARCH PROJECTS

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

(Collaboration with IISc, Bangalore; **Duration:** August 2019 to Aug 2023)

Ravi Kumar G[§], Tulsi Naik K S, Upendra Nongthomba*, K. Vijayan[#]

[§]CSB-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.

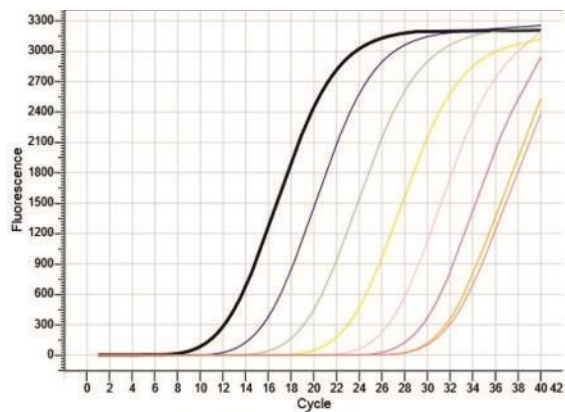
Standard curve generation for qPCR analysis of β -tubulin' copy number in the infected sample

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 that is conserved across the *Nosema* spp. The *N. bombycis* genomic DNA was isolated from the infected samples and the genomic DNA was amplified using β -tubulin, gene specific primer. 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 i.e., 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. Quantification methods. (i) SC method.

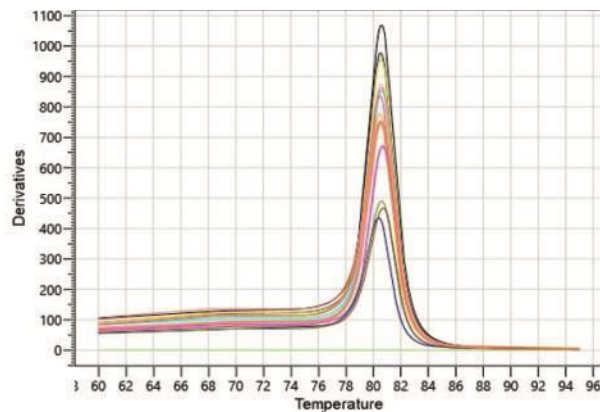
The SC method is widely used in environmental microbiology and employs a dilution series of a defined target concentration. The target concentration (c_{target} [copies μl^{-1}]) was calculated from the DNA concentration (c_{DNA} [ng μl^{-1}]), the lengths of the DNA fragments (l_{DNA} [bp]) (e.g., genome size), the number of targets per DNA fragment (n_{target} [copies]), the Avogadro constant (N_A) (6.022×10^{23} bp mol^{-1}), and the average weight of a double-stranded base pair (M_{bp}) ($660 \text{ g mol}^{-1} = 6.6 \times 10^{11} \text{ ng mol}^{-1}$) (equation 1).

$$\frac{\text{Amount (ng)} \times 6.022 \times 10^{23}}{\text{Length(bp)} \times 1 \times 10^9 \times 660}$$

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 10^7 copies to 10^1 copies was prepared to generate a standard curve. The results of the standardized method for the copy number estimation using SYBR green chemistry is as follows.



Amplification Plots



Melting Curve

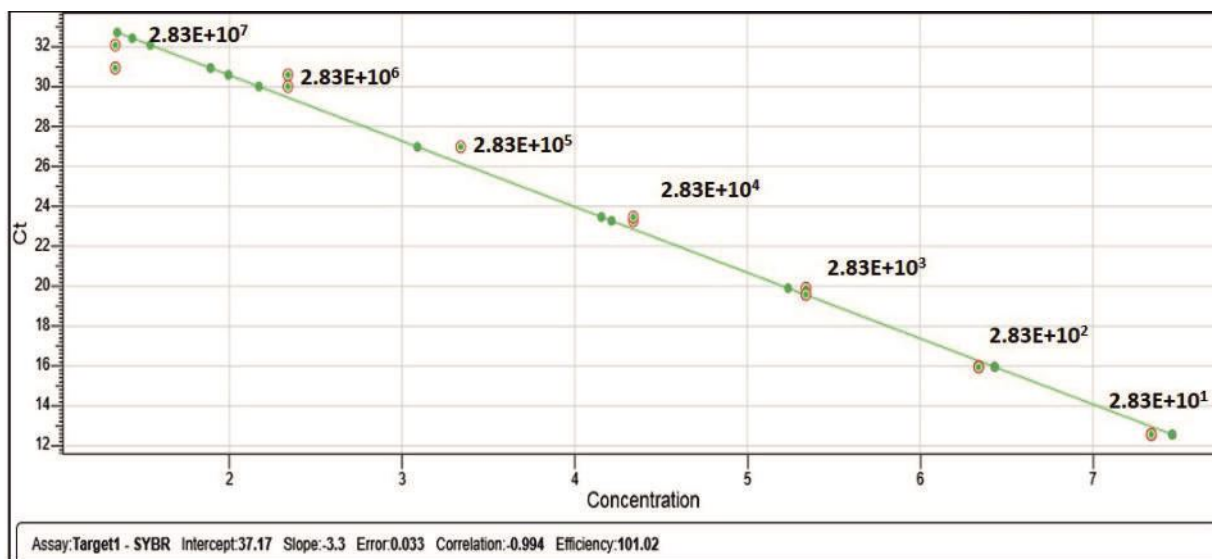


Figure 1: Standard curve for the analysis of β -tubulin gene copy number for the determination of multiplication of *N. bombycis* in silkworm.

The standardized 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 10^1 copies of beta tubulin in the infected samples (**Fig. 1**).

qPCR analysis of β -tubulin' copy number at different time points 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.

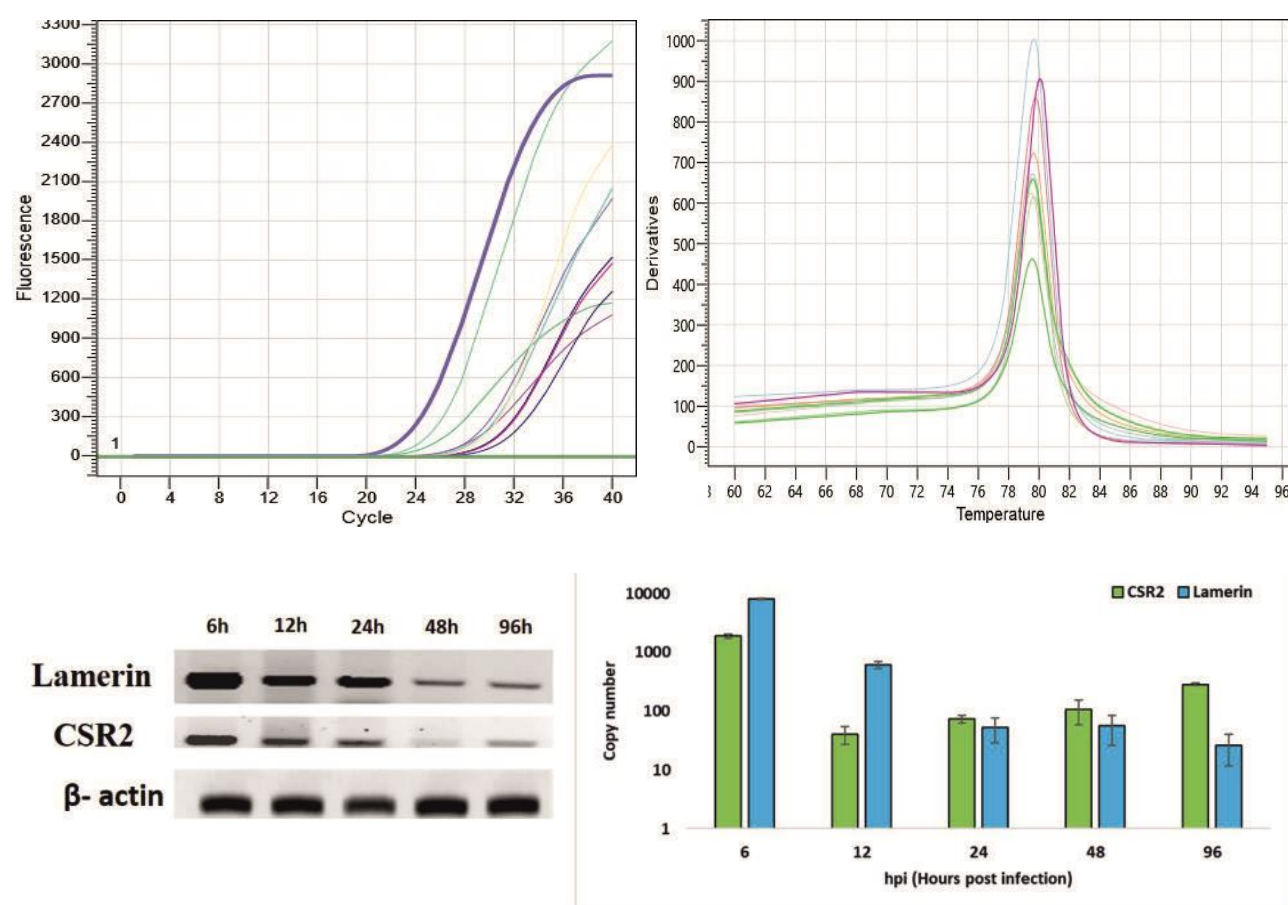


Figure 2: qPCR analysis of multiplication of *N. bombycis* in CSR2 and Lamerin at different time points.

From the qPCR results it can be observed that the multiplication of *N. bombycis* in the Lamerin breed gradually decreased from 6hpi to 96hpi, but in CSR2 it was observed that at 6hpi the multiplication was comparatively less and increased from 48hpi to 96hpi. It is also observed that

though at 6hpi the multiplication was initiated but from 12hpi there was significant decrease in the multiplication of *N. bombysis*. This may be due to the immune response genes that are activated via the Toll and IMD pathway and also response from AMPs of the host against the pathogen. From the results, it can be concluded that though initially the pathogen multiplication is higher at 6hpi, in both the breeds, gradually there was a significant difference in the rate of multiplication of *N. bombysis* in both the breeds. Further, at the 96hpi the >15 copies of *N. bombysis* was observed when compared to > 1000 copies at 6hpi in the Lamerin breed which was evident from the qPCR results (Fig. 2).

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

In this present experiment, we have analyzed 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 the microsporidian *N. bombysis* (Fig. 3). 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 in 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 β 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 Nitric oxide is a reactive free radical mediating in neurotransmission, antimicrobial and antitumoral activities, the expression of Nitric Oxide Synthase 2(*NOS2*) 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. 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.

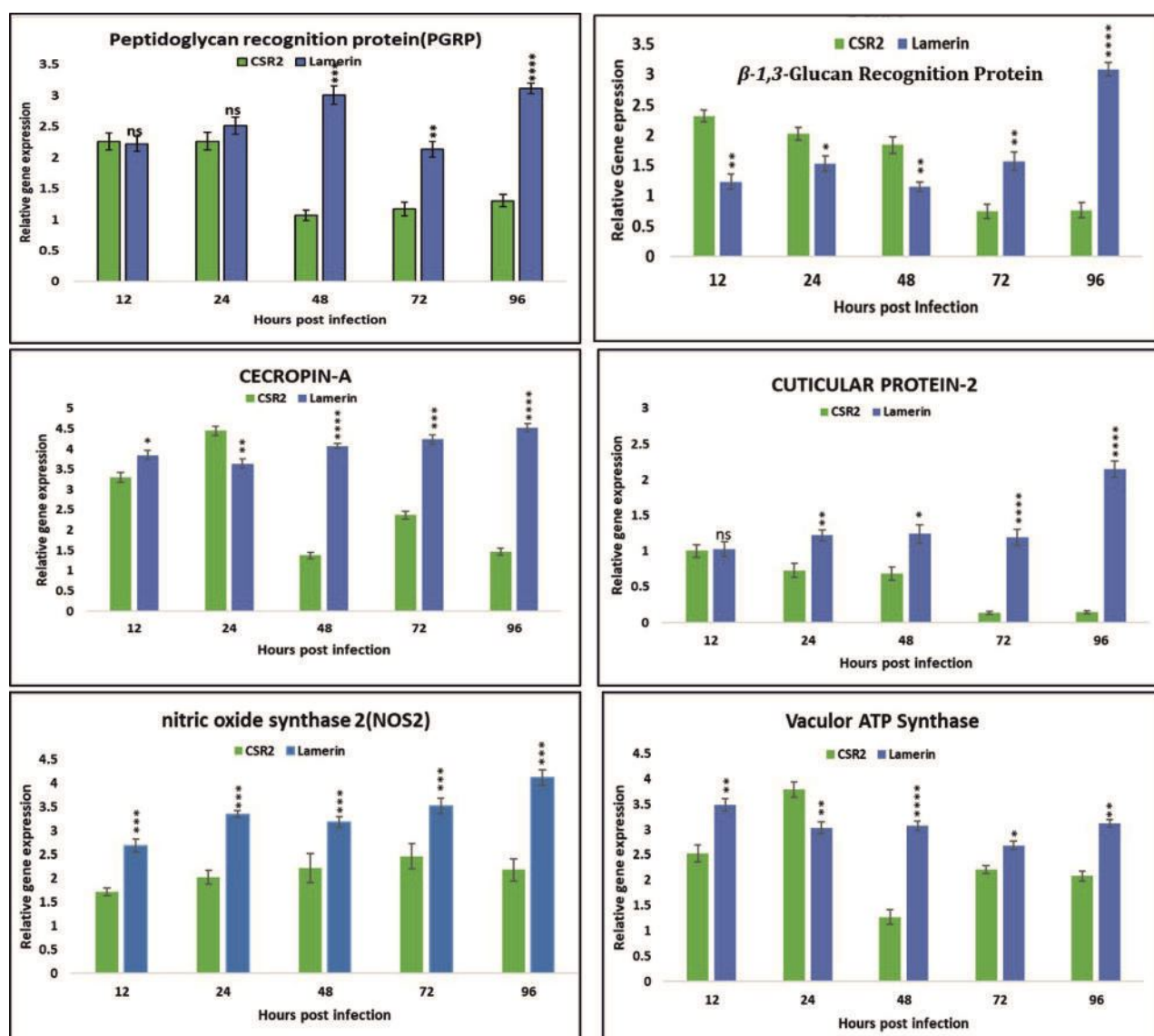


Figure 3: Relative expression of genes in CSR2 and Lamerin infected with *N. bombycis* at different time points.

These results indicate the difference among the breeds in the response against the invading pathogens and another important observation is that the expression of immune genes is significantly higher in the lamerin breed mostly after 48hpi indicating activation of the silkworm systemic immune response. 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.

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

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

Duration: March 2021- January 2023)

K. M. Ponnuvel, G Subrahmanyam, Himanshu Dubey, H S Gadad¹, & Sailaja Bandam², Shama Bhat³

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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.

Methods

Antibody generation

Polyclonal antibodies against *Nosema* SWP1 were generated in 12 weeks old rabbit. For this, rabbit was immunized with approximately 250 µg of purified proteins. 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). Recombinant SWP1 as well as Pebrine infected silkworm samples were used as antigen in ELISA. 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. 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 (**Fig. 4**). 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 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.

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 was noticed (**Table 1, 2**) with both polyclonal and monoclonal antibodies. The recombinant SWP1 shows high binding for polyclonal anti-rabbit SWP5 antibodies even at low concentration. Even at 1:1,28,000 dilution, significant titre is observed (0.6), whereas 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 titers 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. These experiments were 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 using polyclonal and monoclonal antibodies (**Fig. 4**). BSA and in-house P764 protein serves as negative control in the experiment.

Preliminary experiments with LFA kit shown positive results with *Nosema bombycis* infected silkworms samples in which colour development was seen in the form of two bands on LFA pad (**Fig. 5**).

Table 1: 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 2: 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	0.0178

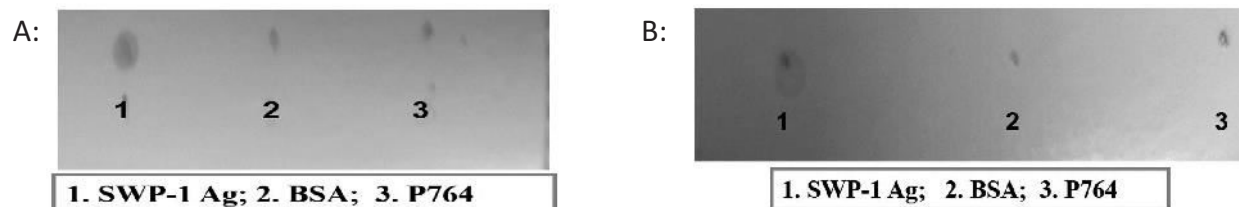


Figure 4: Dot blot assay of SWP1 using monoclonal (A) and polyclonal antibodies (B).

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 (Fig. 5). 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) Further, the developed technology was demonstrated in CSB units and to seri- stakeholders such as silkworm rearers and Chawlki centres.

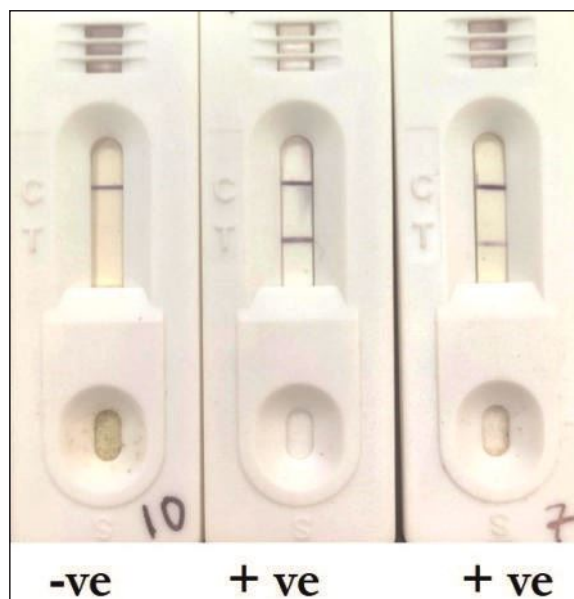


Figure 5: LFA kit developed for the detection of *Nosema* infection in silkworms. Colour development was seen both in negative samples (single band) and *Nosema* infected positive samples (two bands).

3. 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, K. Indira Kumar*, Abhishek Singh[#]

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[#]CSB-P3 Unit, 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

The electrophoretic migration patterns of *Antheraea assamensis* cypovirus (AaCPV) dsRNA revealed 11 discrete bands. A comparison of the electrophoretic patterns showed that AaCPV belonged to type 4 and has closely related to *A. mylitta* cypovirus (AmCPV4) and *A. pernyi* cypovirus (AprCPV). Genomic characterization of AaCPV segment 2 encoding RNA dependent RNA polymerase (RdRp) confirmed the virus as a cypovirus-4 of the family Reoviridae. Total length of RdRp gene is found to be 3797 bp; CDS: 30 to 3737 (3708 bp). Total protein length is of 1235 amino acids with a molecular weight 141.38 kDa. The NCBI accession no for the full-length RdRP gene is ON352655. Comparative genomics showed that the nucleotide sequences and deduced amino acid sequences of the newly identified AaCPV had high levels of identity with those of reported CPV-4 of the same type, but not with CPVs of different types. Conserved motifs were also identified at the ends of each RNA segment of the same virus type. Conserved terminal sequences at 5' and 3' are found to be "ATTAC and TAGAGC" respectively.

Our results are consistent with classification of CPV types based on the electrophoretic patterns, sequencing of structural, non-structural genes and analysis of conserved terminal non-coding regions of CPV double-stranded RNA. Further, *polH* gene encoding polyhedrin protein of AaCPV-4 was cloned in pJET 1.2 blunt vector. The cloned product was sub-cloned in pET32A vector and successfully expressed in *E. coli* BL21 Codon+ cells (**Fig. 6**). A polyphasic protocol for the taxonomic identification of cypoviruses infecting lepidopteran insects was developed in the study. The infectivity of viral pathogen, AaCPV-4 is highly specific to Muga silkworm. Cross transmission studies revealed that the AaCPV-4 did not infect *B. mori*, *Samia ricini* silkworms and other lepidopteran insects such as *Spilosoma obliqua*, *Achaea janata*, *Spodoptera littoralis*, *S. litura* and *Ariadne merione*. Further, a multiplex PCR assay was developed in this study is highly specific for the simultaneous detection of disease-causing pathogens of *A. assamensis* such as *Nosema* sp., and AaCPV-4. Multiplex PCR, was performed using two different primer pairs targeted for β -tubulin genes of *Nosema* sp., RdRp genes of CPV which showed discrete pathogen-specific PCR products (**Fig. 7**). The assay yielded a 268 bp fragment from *Nosema* sp. and 434 bp fragment from AaCPV. The sensitivity of the individual PCR was found to be 100 gene copies for CPV and 1.8×10^2 gene copies for

Nosema. The developed technology was successfully validated through field samples of *Antheraea assamensis*. Results showed that Multiplex PCR is a valuable addition to the diagnostic and surveillance methods in Muga Sericulture.

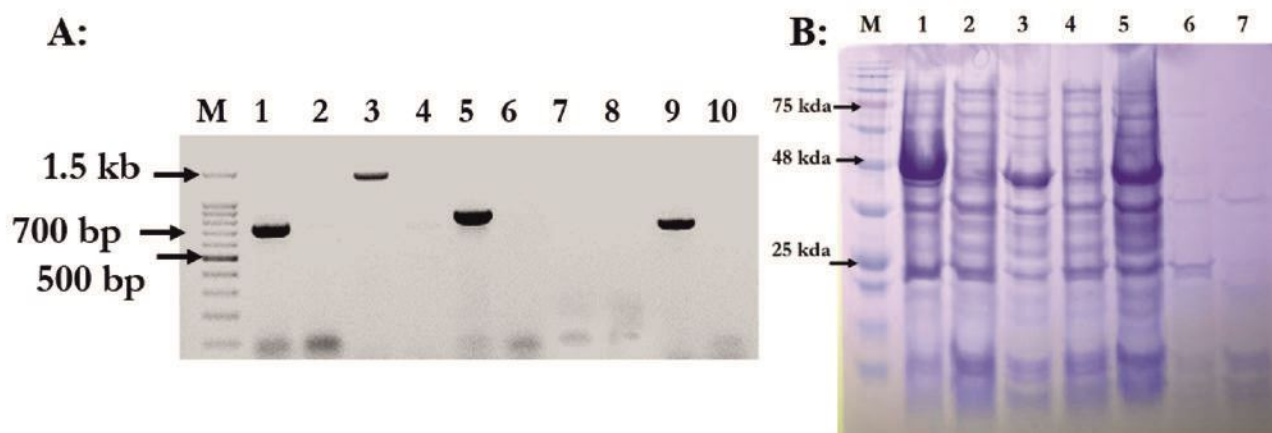


Figure 6: Heterologous expression of AaCPV4 polH gene encoding polyhedrin protein. **A:** Amplification of polH gene. 1: polH Gene specific F (GSF) and R (GSR) (714 bp); 2: NTC; 3: T7 primers F and R (1425 bp); 4: NTC; 5: GSF and vector specific reverse (VSR) (845 bp); 6: NTC; 7 & 8: GSR –VSR; GSF-VSF; 9: T7 primers without insert (711 bp). **B:** SDS-PAGE analysis of AaCPV polyhedrin protein. Marker (245kDa); 1: Positive control –AnprNPV Polyhedrin; 2- Control uninduced AaCPV polyhedrin; 3: Induced AaCPV Polyhedrin; 4- Control uninduced AaCPV Polyhedrin; 5: Induced AaCPV Polyhedrin; 6: Induced supernatant; 7: Induced supernatant AaCPV Polyhedrin.

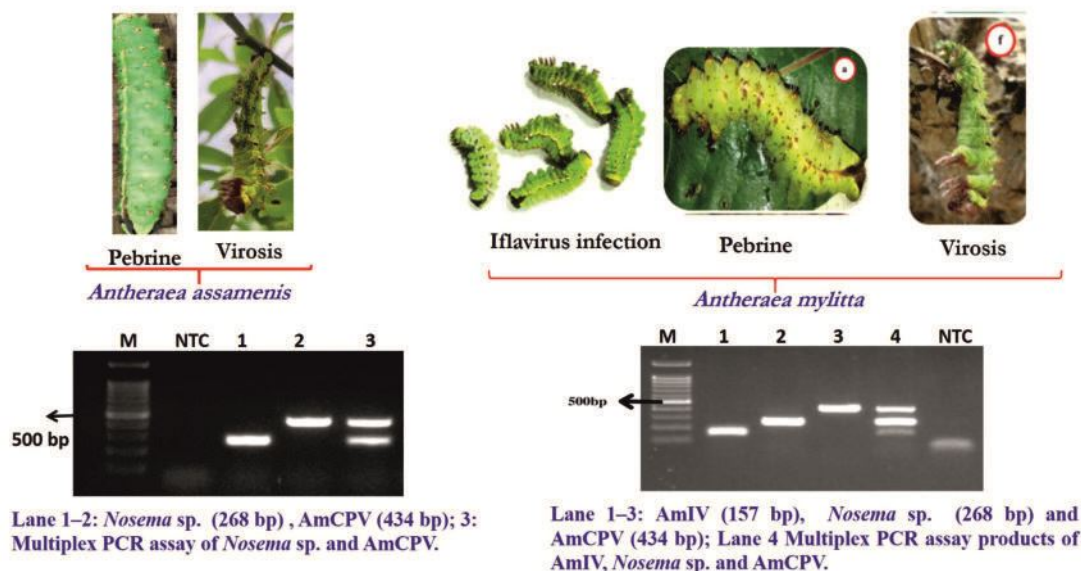


Figure 7: Multiplex PCR developed for the simultaneous detection of Pebrine, cyrovirus (CPV) and iflavirus infecting *A. assamensis* and *A. mylitta* silkworm.

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

(Collaboration with CSB-CMER&TI, CSB-SSTL, CSB-P2-BSF-Topatoli & CSB-ESSO-Hosur;

Duration: March 2022-February 2025)

R. Debnath, M. Moorthy¹, Ramesha A, Himanshu Dubey, Malashree N², L. Natarajan³ & P. Kumar⁴

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

Eri silkworms (*Samia ricini*) with distinct body colorations/patterns are valuable germplasm resources and are important in preserving genetic diversity. The variations can be used to study the underlying genetics, which can lead to the development of improved breeds.

In this newly initiated study, as part of the first objective to obtain stable breeding lines of eri silkworm morphotypes; mixed population of eri silkworms from populations of Borduar/Titabor ecorace were used as starting populations for developing lines of eri silkworm distinguishing solely based on morphotype. Experimental rearings are conducted both at SBRL and ESSO, Hosur simultaneously by feeding on *Ricinus communis* (castor) leaves (**Fig. 8**). Triplicate cellular rearings were conducted at each subsequent generations during the year. Based on body colouration and integument markings, lines of yellow plain (YP), greenish blue plain (GBP), yellow zebra (YZ) and Greenish blue zebra (GBZ) are being developed by repeated sib-mating and selection until non-segregating lines are obtained (**Fig. 9**).



Figure 8: Rearing activities; **a.** Collaborators of P2-BSF Topatoli at SBRL; **b.** SSTL, Kodathi; and **c.** ESSO Hosur

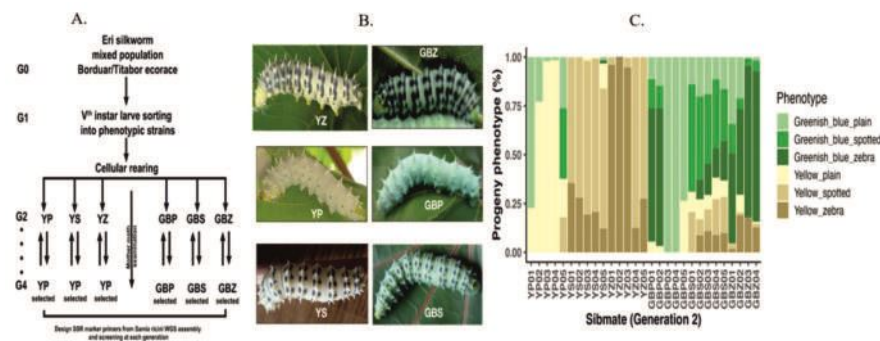


Figure 9: A) Schema of the rearing workflow for generating stable morphotypic lines of eri silkmoth. B) Four different morphotypes of eri silkmoth YP, YZ, YS, GBP, GBS, GBZ. C) Stacked bar plot of progeny proportions (%) in G2 generation from inbred morphotypes showing segregating populations

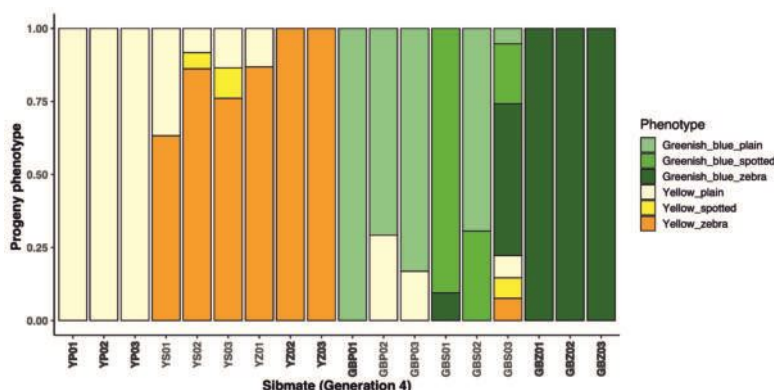


Figure 10: Observation of partial homogeneity in larval phenotype of four lines YP, YZ, GBP, GBZ in sibmated G4 generation.

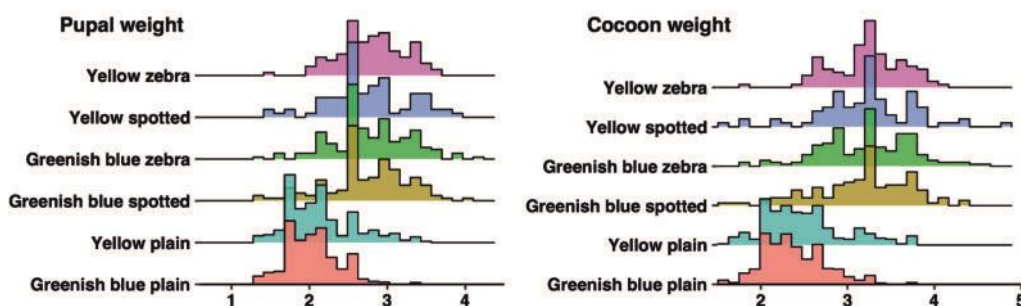


Figure 11: Distribution of cocoon and pupal weight of the morphotypic lines at G4 generation. The median weights of both cocoon and pupa for the spotted and zebra lines in both body colour yellow and greenish blue were higher than YP and GBP ($p < 0.05$)

All the lines are being maintained and reared till cocoon formation and grainage operations. Mother moth examination was carried out at each generation. Only initial 02 days layings were propagated for the subsequent generations to avoid non-synchronous hatching. At G4 generation partial homogeneity for larval morphotype were observed for plain and zebra morphotypes for both body colour phenotypes (Fig. 10). The rearing parameters scored for the separate lines show higher median values for cocoon and pupal weight in zebra lines of both yellow and greenish blue body colour (Fig. 11). Further rearing experiments are continued and SSR markers are developed from insilico analysis of *Samia ricini* genome assembly.

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

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

Pawan Shukla, Ramesha A, Himanshu Dubey, Mahiba Helen¹

¹CSB-CSR&TI, Mysore

Objective:

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

Like other crop plants, several insect pests attack mulberry and cause severe damage to mulberry leaves which in turn adversely affect the mulberry leaf yield. Among the major pests of mulberry, the pink mealy bug *Maconellicoccus hirsutus* (Green) is considered as a serious pest which like other crop plants, several insect pests attack mulberry and cause severe damage to mulberry leaves which in turn adversely affect the mulberry leaf yield. Among the major pests of mulberry, the pink mealy bug *Maconellicoccus hirsutus* (Green) is considered as a serious pest which damaged the tender leaves leads to TUKRA disease. The average incidence and loss in mulberry leaf yield in southern sericultural belt are estimated to be 34.24% and 4500 kg/ha/yr, respectively. During feeding, mealybug injects toxic saliva into the plant that results in malformed leaf and shoots growth, stunting, and occasional death. Heavily infested plants have shortened internodes leading to resetting or a "bunchy top" appearance. A heavy, black, sooty mold may develop on an infested plant's leaves and stems as a result of the mealybug's heavy honey-dew secretions.

Insecticides fail to control this pest, because they cannot penetrate the heavy layers of wax that shield the insect body. The egg stage, in particular, is protected by a white, waxy ovisac (Kairo et al., 2000). Biological control, using predators and parasitoids, can be an effective strategy to control mealybug populations. Its use has increased during the last decades in several crops. However, the success of parasitoids and predators in controlling mealybugs can be counteracted by the presence of ants, which has mutualistic association with the pest, ants obtain honeydew (carbohydrates) by tending mealybugs and, in return, they improve hygiene of the mealybug colony (when ants remove dead individuals or exuviae), transport them to new feeding sites and, more importantly, protect them against natural enemies. Ants have disrupted several biological control programs against mealybugs by disrupting, attacking or killing parasitoids and predators. Therefore, an alternate strategy is required for effective control of mulberry mealybug. In this backdrop, the project is aimed to understand mealybug outer wax, salivary proteins and honeydew composition for devising the strategy to control the mealybug in mulberry which in turn helpful in managing tukra disease.

In the current project, mealybug rearing has been established at SBRL, Kodathi (**Fig. 12**). Honeydew has been collected (**Fig. 13**) for GC-MS analysis. In order to perform protein profiling, salivary glands of mealybug were isolated (**Fig. 14**) and collected in SDT buffer and stored in -20 °C for further analysis.



Washing

Inoculation

Grown mealybug

Figure 12: Establishment of Mealybug rearing at SBRL, Kodathi



Figure 13: Honeydew collection from Mealybug

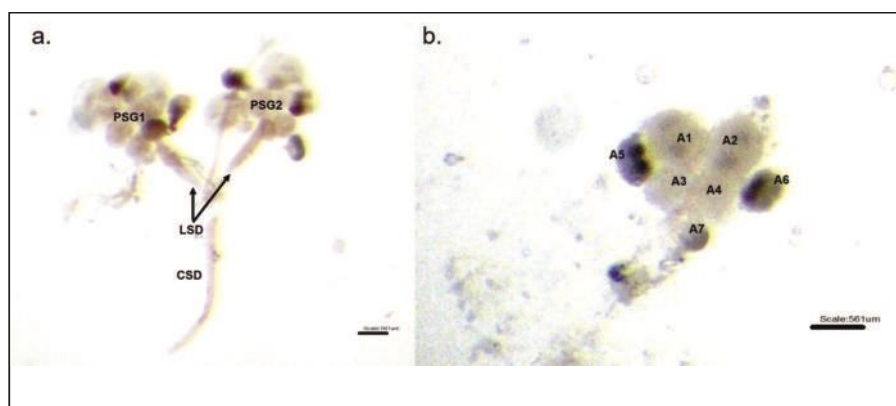


Figure 14: **a.** Salivary glands of the pink mealybug; **b.** Arrangement of acini's in each principle salivary gland, PSG: Principle Salivary Gland, LSD: Lateral Salivary Duct, CSD: Common Salivary Duct and A: Acini (Scale Bar: 561 μ m)

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

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

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

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

Objectives:

1. Analysis of small RNA mediated modulation in immunity during favorable and unfavorable rearing seasons of muga silkworm.
2. Development of molecular markers associated with disease susceptibility in muga population.

Muga silkworm (*Antheraea assamensis* Helfer) is a wild insect found primarily in North-Eastern states such as Assam, Meghalaya, and Arunachal Pradesh. Unlike the domesticated *Bombyx mori* silkworm, Muga silkworm is challenging to rear in captivity due to its wild nature. It undergoes up to 6 rearing cycles annually, including commercial, pre-seed, and seed crops, relying on host tree plants like Som (*Persea bombycina*) and Soalu (*Litsaea monopetala*) in outdoor conditions. The optimal rearing conditions are 20-31 °C temperature and 65-75% relative humidity. Continuous rearing of Muga silkworms is essential for seed production, but it is hindered by unfavorable environmental conditions, leading to high larval mortality and affecting seed availability. Muga silkworm is highly susceptible to changing environmental conditions, which can promote the growth of pathogens and increase larval mortality. However, the molecular mechanisms underlying these changes are not well understood. The study aims to investigate the impact of environmental conditions on gene expression in Muga silkworms. Transcriptomic analysis, including mRNA and miRNA analysis, will be performed. A record of larval mortality in different rearing seasons is maintained along with temperature and humidity. Rearing of Muga silkworm was conducted at MESSO units located in Rompara and Nongpoh Meghalaya. In the Nongpoh unit, rearing was conducted for Chotua, Baisakhi, Aherua, and Bhodiya seasons, while in Rompara, rearing was conducted for Baisakhi, Bhodiya, and Aherua seasons. Preliminary observations suggest that pest and disease incidence is higher in Baisakhi and Bhodiya seasons compared to the Aherua season in Rompara unit. However, disease incidence is generally lower in all rearing seasons in the Nongpoh unit compared to the Rompara unit. Nonetheless, a small incidence of Flacherie was observed in the Bhodiya season in the Nongpoh unit (**Fig. 15**).

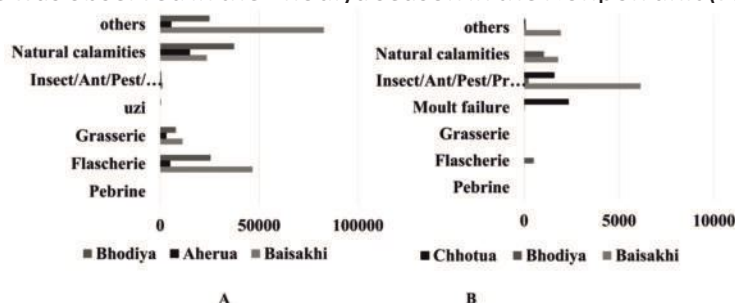


Figure 15: Incidence of pest and diseases in three seasons of muga silkworm rearing at (A) Rompara, and (B) Nongpoh Meghalaya

7.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, Tulsi Naik, AR. Pradeep¹

¹CSB-CSR&TI Berhampore

Objectives:

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 authorised at farmers level in West Bengal and north-west and North-east India against the existing ruling hybrids.

A total of 92750 nos of MASN4xCSR4 (bivoltine hybrid) DFLs and 25650 DFLs of Nistari x MASN4 (Cross breed) were tested in East and North-West during 2018-2020 in a concluded project ARP 3605. In the current project, rearing 2.0 lakh DFLs of MASN4xCSR4 will and 2.75 lakhs DFLs of Nistari x MASN4 will be tested at locations distributed in North, and North West. Nistari x MASN4 will be tested at West Bengal and Tripura states. MASN4 x CSR4 (bi x bi) will be tested at Jammu, Uttarakhand and Himachal Pradesh. MASN lines were maintained through continues rearing. A total of 600 P1 MASN DFLs were produced for further multiplication at NSSO centers.

OUTCOME OF CONCLUDED RESEARCH PROJECTS

1. PRP08002MI: Identification of powdery mildew resistant genes and validation of CAPS marker for Chalcone synthase in mulberry Spp. Caused by *Phyllactinia corylea* (Pers.) Karst.

(Collaboration with CSB-CSR&TI, Berhampore; Duration: May 2019- May 2022)

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

*CSB-CSR&TI, Berhampore; #CSB, Bangalore

Among the mulberry diseases, powdery mildew is one of the major foliar diseases, which negatively affect quality and quantity of mulberry leaf available to produce silk. For disease resistance breeding, deployment of susceptibility (S) genes is an interesting approach compared 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 some crops to counter the powdery mildew fungi infection.

Objectives of the project are

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

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.

Alternative splicing of candidate *MLO* genes

Loss of function of susceptibility genes such as *MLO* genes results in powdery mildew disease resistance in plants. It has been shown in many crop plants that either natural mutations or induced mutations through RNAi or genome editing of candidate *MLO* genes results in powdery mildew resistance. To check whether tolerant mulberry genotypes present in the germplasm have non-functional mutations in the identified candidate *MLO2* and *MLO6A* genes. Total RNA and cDNA from tolerant mulberry accessions for powdery mildew were isolated under infection conditions. *MLO2* and *MLO6A* full-length coding sequences were amplified, sequenced and compared with Heyebai genotype from *Morus alba*, where good quality genome sequence data is available in public domain.

Coding sequence of *MLO2* from ME-0260 was sequenced and aligned to reference *MLO2* coding sequence. In one of the clone 4th intron is retained and stop codon is present in the 4th intron resulting in truncated *MLO2* protein. In another clone, 9th and 10th introns were retained that resulted in truncated *MLO2* protein (**Fig. 16**). In addition to intron retention clones, properly spliced out and forming full length protein was also obtained. This implies that the proportion of *MLO2* protein

produced in ME-0260 could be non-functional. In ME-0267 it was observed that part of the intron 11 is retained and remaining exons, 12,13 and 14th exons were skipped in the coding sequence resulting in truncated protein lacking C-terminal region of the protein. In MI-0028 intron 9, intron 10 and partly intron 11 is retained and exons 12, 13 and 14th were skipped. The insertion of introns and exons skipping resulted in truncated MLO2 protein. Similarly, the coding sequences of MI-0125 was sequenced and found that intron 9, intron 10 and partly intron 11 is retained and exons 12, 13 and 14th were skipped. The insertion of introns and exons skipping resulted in truncated MLO2 protein.

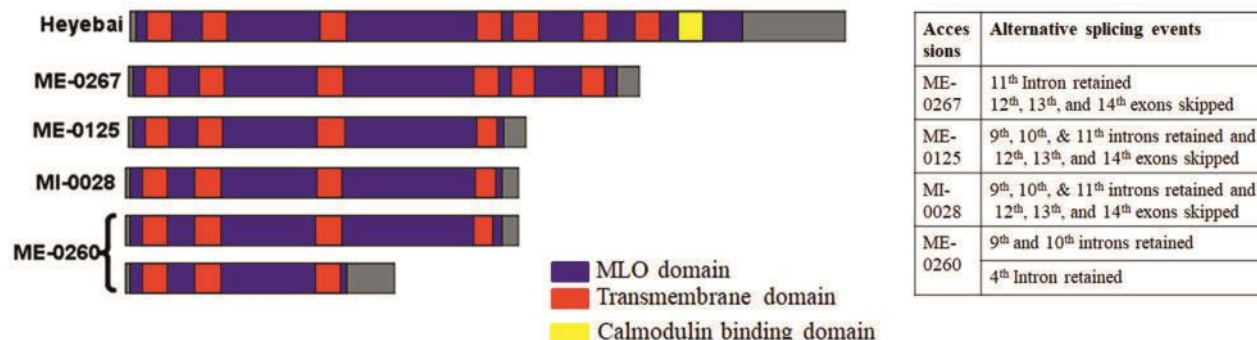


Figure 16: Representation of alternative splicing of MLO2 gene in tolerant mulberry accessions to powdery mildew resulting in truncated protein.

A total of 21 accessions/species, RFS175, C176, S1, *M. indica*, BR-8, Punjab Local, Himachal Local, AGB-8, Assama Bola, S34, S146, S1635, DD, AR12, Thailand Male, S13, V1, *M. rotundiloba*, *M. serrata*, *M. multicaulis* and *M. laevigata* re-sequenced data was retrieved from NCBI. The K2 variety sequence used as a reference sequence and point mutations in different accessions was identified. The mutations observed were in heterozygous condition. The detail mutations observed in different mulberry accessions in clade V genes falling in important domains are represented in **Fig. 17**.

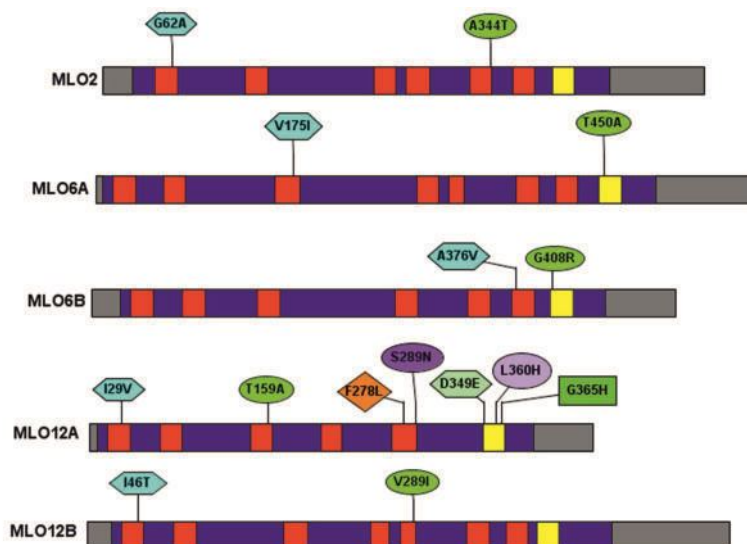


Figure 17: Representation of mutations in clade V genes in different mulberry accessions in comparison to reference MLO2 from K2 variety. Re-sequencing data was analysed to identify the mutations. The mutations falls in the important domains were displayed.

Validation of chalcone synthase CAPS marker with the powdery mildew tolerance

Chalcone synthase (CHS) CAPS marker association with powdery mildew tolerance was analysed in mulberry germplasm and segregating progenies derived from Kajali-OP X V1 and S1 X Vietnam-2. The established segregating progenies from Kajali-OP X V1 and S1 X Vietnam-2 were phenotyped at CSR&TI, Berhampore in three powdery mildew disease seasons of 2019, 2020 and 2021. The Kajali-OP and Vietnam-2 are tolerant to powdery mildew and V1 and S1 are susceptible to powdery mildew disease. The Chi-square analysis of CHS_CAPS marker genotype with powdery mildew phenotype from segregating progenies revealed that there is no statistically significant association of CHS_CAPS marker with the powdery mildew tolerance phenotype. However, CAPS marker homozygous genotype plants were skewed towards powdery mildew tolerance and heterozygous genotype plants were skewed towards susceptible phenotype (**Fig. 18**).

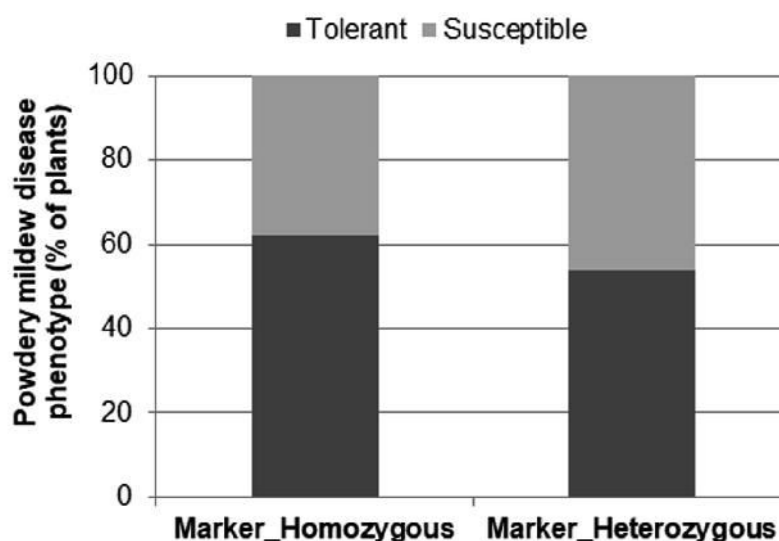


Figure 18: Percentage of powdery mildew tolerant and susceptible plants showing homozygous and heterozygous genotype based on CHS_CAPS marker.

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

(Collaboration with CSB-CSGRC, Hosur; **Duration:** March 2020- March 2023)

A. Ramesha, Himanshu Dubey, Raju Mondal* and Prashanth Sangannavar[#]

*CSB-CSGRC, Hosur, [#]CSB, Bangalore

To increase the quality and quantity of mulberry leaf production through breeding, understanding genetics of agronomic and economic traits is important. Homozygous/inbred lines are used for generating gold standard reference genome, 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. Therefore, this project aims to standardize the protocol for haploid embryogenesis/callus formation.

Objective

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

Androgenesis by anthers and microspore culture did not yield the desired results of obtaining haploid embryos/callus, therefore attempt was made to generate haploid embryos through the gynogenic route. In this method, we used un-pollinated ovary/ovule from the female catkin producing mulberry accessions such as G4, G2 and other accessions. We observed that ovary and cut opened ovary response in terms of enlargement and growth of tissue was good (80%). But, when dissected ovule was cultured, response was weak, which was around 20% (**Fig. 19**). When complete ovary was cultured, growth was observed from the ovary walls but not from the ovule, which is undesirable with outcome of haploid embryo formation. The cut opened ovary culture resulted in good response and growth and formation of callus was observed from the ovule (**Fig. 20**). Since the response from the cut opened ovary was good and also growth and development of callus was observed from the ovule, subsequent experiments were performed with cut opened ovaries.

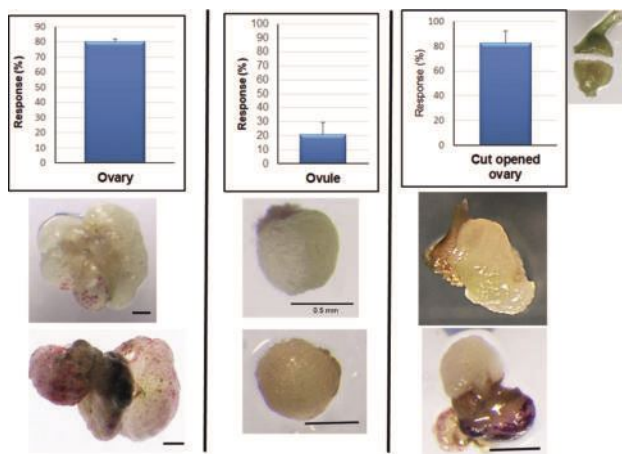


Figure 19: Response (%) and growth and development of cultured un-pollinated ovary, ovule and cut opened ovary as explants.

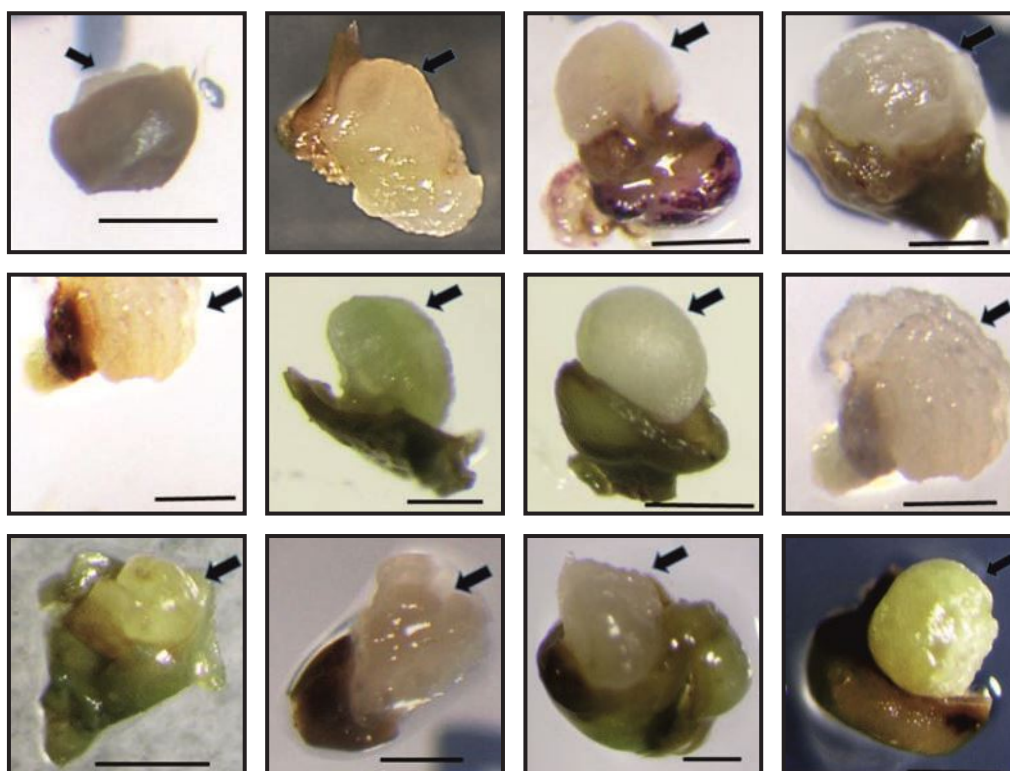


Figure 20: The growth and development of cultured cut opened ovary as an explants from G4 variety

The available genome sequence of *M. alba* cultivar, Heyebai (assembly ASM1206604v2) was downloaded from NCBI genomes database on January, 2021 was utilized for mining microsatellites markers with different types of repeat motifs from di- to hexa-nucleotide. A total of 55,148 SSRs with minimum motif length of ≥ 20 nt, were identified. Twenty eight single locus SSRs were tested with G4 donor variety as an explant. The SSR markers were selected based on the heterozygous nature of the SSR locus, which gives two bands in the G4 variety. The SSR markers, M1, M8, M10, M22, M23, M24, M27 and M29 gives double band stating that these loci are in heterozygous condition in G4 variety (**Fig. 21**). Difficulties associated with the recalcitrance and low frequency of haploids required high throughput, simple and cost effective diagnostic methods. SSR markers are simple and co-dominant in nature and highly suits for identification of haploids and doubled haploids.

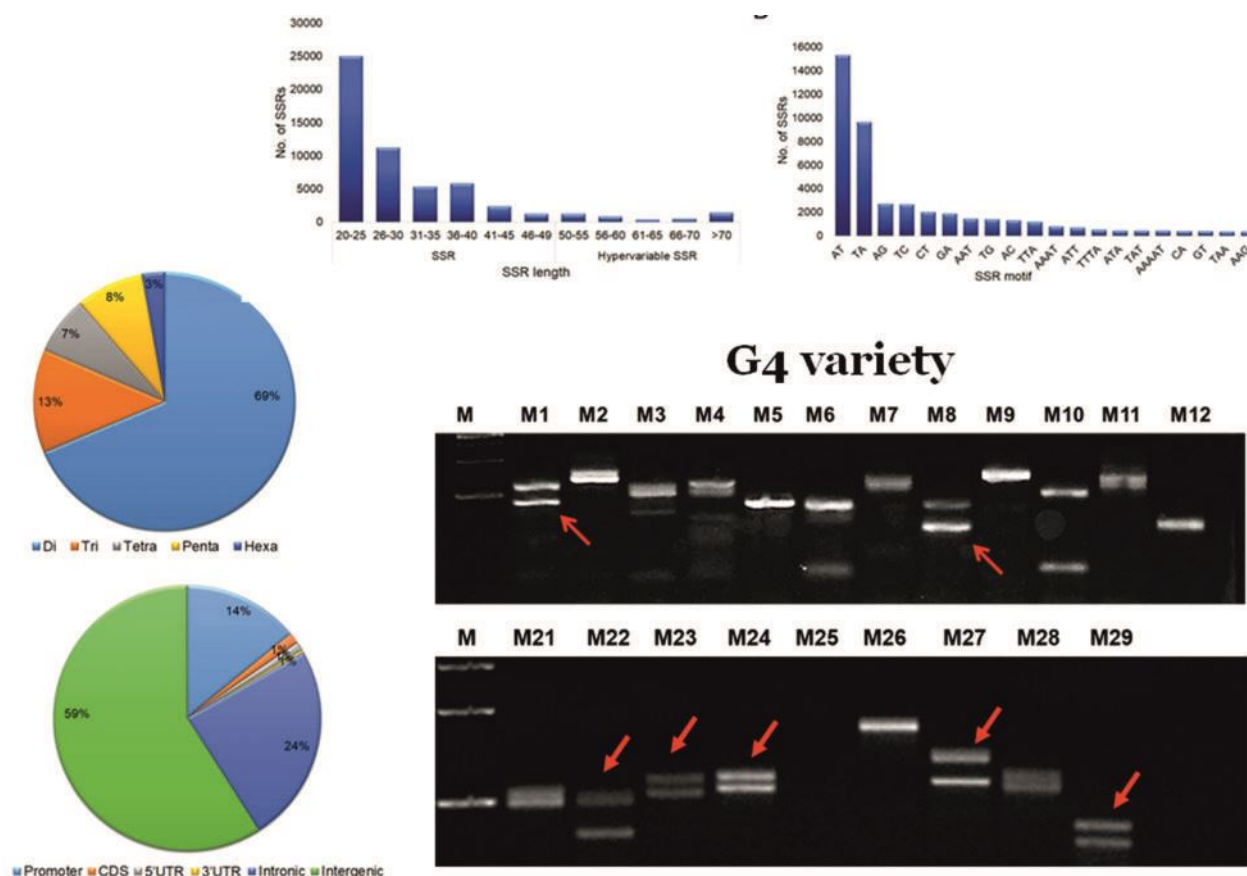


Figure 21: Simple sequence repeats (SSR) characterization with number of repeats, type of repeats and location of SSR in the genome. Single locus SSR primers were designed to amplify in G4 variety. The SSR markers giving double band (heterozygous nature) were selected for ploidy analysis from the tissue culture callus/plants.

G4 variety female un-pollinated catkins were collected and cold stress was imposed by incubating at 4°C for 3 days. The cut opened ovaries (Half ovary) were cultured on MS Media (Full Strength) + 3% sucrose + 0.8% Agar + BAP (1 mg/l). The Plates were covered with aluminium foil to provide dark condition and kept in incubation at 35 °C for 3 days and later transferred to 25 °C in culture room. After fifteen days of culture, one plantlet emerged directly by direct embryogenesis. After one month, the plantlet was transferred to MS media (Full Strength) + 3% Sucrose + 0.8% agar + BAP (1 mg/l) + GA3 (1.5mg/l). To check the ploidy of the emerged plantlet, we isolated genomic DNA from the small leaf. SSR markers (M8, M14, M22, M23 and M28) giving double band in G4 variety were selected for checking ploidy. The sample 3 (plantlet) in the gel picture (**Fig. 22**) gave single band in all the tested SSR markers, indicating haploid nature of the plant. To further validate the haploid nature of the plant, chromosome count was performed, the sample 3 (plantlet) the chromosome number was 14 corresponding to the haploid chromosome number. Whereas, the

explant, G4 variety the number of chromosomes counted was 28. This clearly indicated that the plantlet obtained was haploid which was confirmed by SSR marker analysis and by chromosome count. When the haploid plantlet was sub cultured, it got contaminated with bacteria. We try to rescue the plantlet with surface disinfection and culturing with antibiotics however, these methods could not rescue and plantlet died with infection.

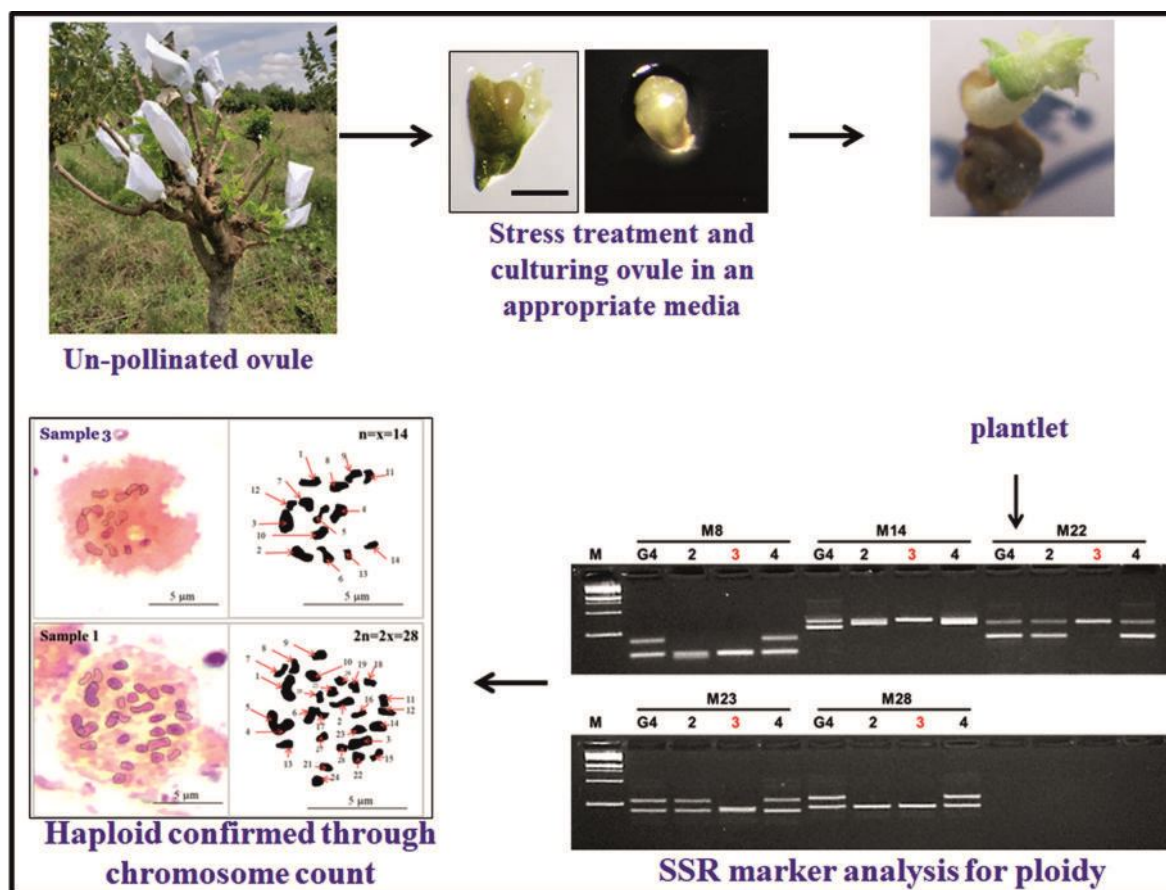


Figure 22: Generation and validation of gynogenic haploid plant in mulberry. The Un-pollinated cut opened ovary was used as an explant to induce direct embryogenesis to produce plantlet. The ploidy was confirmed by multiple SSR markers (sample 3 giving single band indicating potential haploid) and it was further validated by chromosome count showing only 14 chromosomes in sample 3.

This study identified some of the factors contributing to the response of ovule for haploid embryogenesis/callus. Gynogenic method of haploid embryogenesis is responsive compared to androgenic (anther and microspore) method, so that future attempts can be made with gynogenic route rather than androgenic method. Cut opened ovary/exposed ovule culture is responsive than complete ovary and ovule culture. Single locus SSR markers were identified to screen and identify haploid callus/plants.

3. AIT08005MI: Development and Evaluation of Bidsenovirus resistant silkworm hybrids developed from marker assisted breeding lines -Phase II

(Collaboration with CSB-CSR&TI, Mysore & CSB-CSR&TI, Berhampore;

Duration: March 2020- February 2023)

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

K. Rahul[#] & Mihir Rabha[#]

*CSB-CSR&TI, Mysore; [#]CSB-CSR&TI, Berhampore

Objectives

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

The four robust/productive bivoltine double hybrids of silkworm *Bombyx mori* L. developed at SBRL, Bangalore viz., [CSR6R × CSR26R] × [CSR2R × CSR27R] resistant to *BmBDV* infection along with the existing bivoltine single double hybrid in the field [CSR6 × CSR26] × [CSR2 × CSR2R], susceptible to *BmBDV* was used as a control were assessed under large scale in-house evaluation as per the farmers practice (**Fig. 23a & 23b**). Further, 10 DFLs of both resistant and susceptible double hybrids were evaluated at four RSRS stations for three seasons i.e. rainy (July-Oct), Winter/Autumn (Dec-Feb) & summer/Spring (March-May) seasons, by following standard rearing technique on V1 mulberry leaf and evaluated for their various economic traits and most importantly for their survival compared to control under different seasons and locations (**Table 3**). The cocoons were harvested on 6th day of mounting and assessed on multi-end reeling machine at Silk Reeling Unit of CSRTI, Mysore for assessing the reeling parameters. To know the cocoon size variability, one hundred cocoons were randomly picked up and three cocoon size variables viz., cocoon length, cocoon width and length/width index were determined

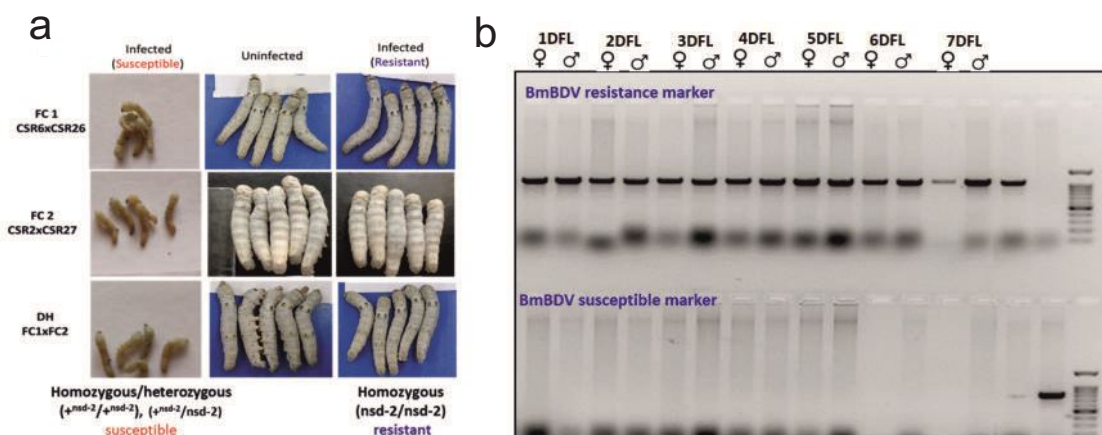


Figure 23:a. Bioassay for homozygous BmBDV resistant and susceptible Double hybrids **b**. Screening individual male and female moths of double hybrids to confirm BmBDV resistance.

Table 3: Data collection from four RSRS stations showing the performance of the breeds at different seasons

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

Development of *BmBDV* homozygous resistant breeds and hybrids of SK6 and SK7 breeds of Berhmapore

To develop *BmBDV* resistant homozygous lines, screening and selection of *nsd-2* resistant allele through marker assisted selection was employed. Out of the 10 selected bivoltine breeds which showed *nsd-2* resistant allele in heterozygous condition, we selected SK6 and SK7 breeds for developing homozygous *BmBDV* resistant lines because of their thermo tolerance, economic traits and as parents of hybrid in tropical conditions. Individual male and female moths pertaining to twenty-five DFLs from each breed i.e., SK6 and SK7 were screened to obtain both male and female moths of DFLs homozygous to *nsd-2* resistant alleles. Out of 25 DFLs that was prepared from the parents in each breed, 8 DFLs (30%) in SK6 breed and 7 DFLs (24%) in SK7 showed homozygous *nsd-2* resistant condition for both the parents. These marker assisted selected breeds were named as SK6R and SK7R as they carried *nsd-2* resistant allele in homozygous condition. The DFLs of those male and female parents that possessed the *nsd-2* resistant allele in homozygous condition were selected and reared to maintain the resistant lines. Once the homozygous lines were prepared in each generation, good quality cocoons were selected and used for post cocoon studies. Since hybrids resulting from crossing SK6 and SK7 are used as parents in hybrid development, we made the crosses between the resistant parents, SK6R x SK7R, SK7R x SK6R and the susceptible parents, SK6x SK7 and SK7 x SK6 to test *BmBDV* resistance in hybrids through per oral infection (Fig. 24a & 24b). Further the same hybrids, i.e., SK6x SK7, SK7 x SK6 and SK6R x SK7R, SK7R x SK6R without infection were also evaluated for the pre-cocoon and the post-cocoon parameters.

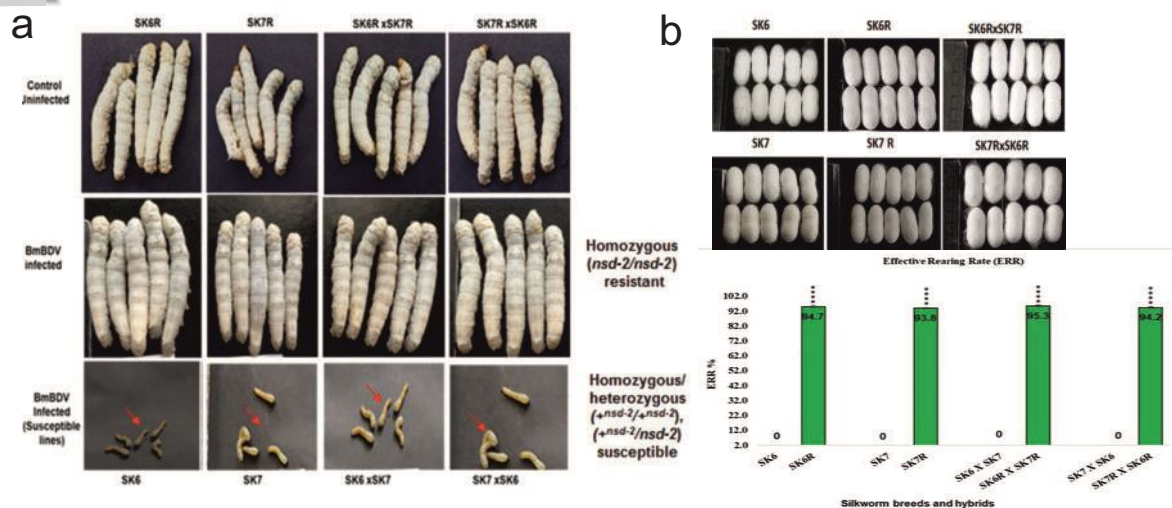


Figure 24: a. Bioassay for homozygous BmBDV resistant and susceptible hybrids b. Cocoon characters and the Effective rearing rate (ERR%) among the control and the resistant breeds and hybrids

Development and evaluation of cross breed hybrids for *BmBDV* resistance

In the present study, 17 multivoltine breeds were screened from West Bengal region of India through marker assisted selection among them only Lamerin, a multivoltine breed showed complete resistance to *BmBDV* infection due to the presence of *nsd-2* resistant alleles in homozygous condition. In earlier studies, it is reported that Lamerin breed is the only breed which survives with associated microsporidian (*Lb_{ms}*) infection from generations (Bhat and Nataraju, 2005). The cocoon characters of Lamerin are very poor but it is resistant to different diseases and pathogens. Similar results were observed which is in conformity with the observations made by (Bhat and Nataraju, 2009). The male and female moths of Lamerin breed were labelled after crossing and tested through conventional PCR with *nsd-2* marker. In our study, we found all the DFLs are in homozygous resistant condition, which was also confirmed by bioassay studies. Hence, our results reveal Lamerin breed is resistant to *BmBDV* virus even though inoculated with high concentration of inoculum. In other experiment, CSR2R bivoltine breed which was developed through backcross method was taken as bivoltine parent for development of cross breed. The female moth of LamerinR was crossed with male moth of CSR2R and DFLs were prepared. The crossed moths PCR results reveal that, all the DFLs showed complete resistance to *nsd-2* resistant allele. To confirm this, first instar larva of F1 hybrid was collected and inoculated with *BmBDV* virus inoculum and checked for the survival rate in triplicates. After 10 days post infection these resistant breeds were compared with control. In this, Lamerin R x CSR2R hybrid did not show any symptoms of *BmBDV* infection and conventional PCR also showed absence of viral genome from midgut tissue at different days after infection.

Further, the mortality rate for *BmBDV* infection was 90% with 10.66% survival rate in infected control breeds while, the resistant breeds showed >90% survivability and <10% mortality due to other infections such as *BmNPV* and improper growth and development. The infected susceptible breeds showed multiple infections with *BmBDV* and *BmNPV*. There was no significant difference in the growth and development of the larvae and cocoons between the infected resistant breeds and the uninfected control batches (Fig. 25a & 25b).

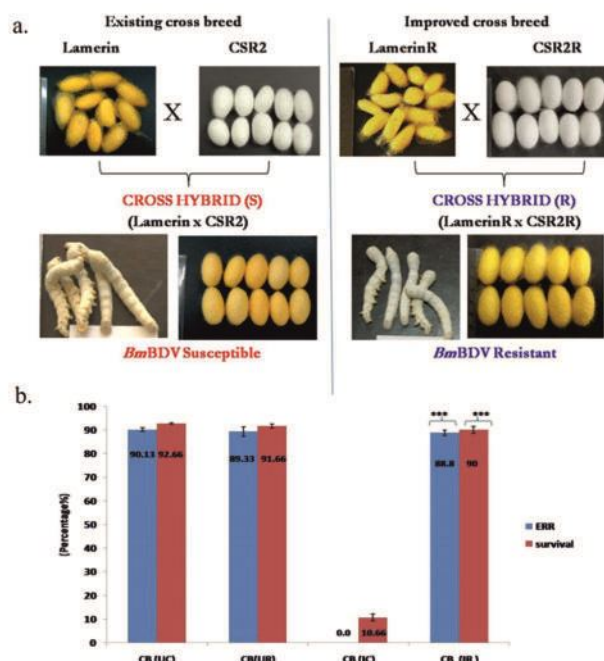


Figure 25: a. Comparison of cocoon shape and colour of *BmBDV* susceptible and resistant cross breeds. **b.** Graphical representation of economic parameters of ERR and survival of the cross breeds in resistant and control batches through two way ANOVA. The significant difference in all the parameters were indicated as * i.e., $p > 0.05$, ** $p > 0.001$, *** $p > 0.0001$. *(UC) - uninfected control, (UR) - uninfected resistant, (IC) - Infected control, (IR) - Infected resistant

Significant findings during the study

- 1) Developed FC1 and FC2 resistant to *BmBDV* (Viral Falcherie) infection. The on-station trials of the Double hybrids (FC1 X FC2) showed minimum of 3% and maximum of 12 % increase in the survivability as well as yield/100DFLs compared to control.
- 2) Out of 20 bivoltine breeds of Berhampore, 10 breeds were found to have *nsd-2* resistant alleles in heterozygous condition, SK6, SK7, BHP8, BHP9 homozygous lines for *BmBDV* resistance were identified and maintained at CSRTI, Berhampore.
- 3) Out of 20 multivoltine breeds, the *nsd-2* resistance alleles were identified in Sarupat, C.nichi and Lamerin breeds (hibernated multivoltine). A homozygous line of Lamerin x CSR2R was developed through marker-assisted selection.
- 4) The Homozygous lines of the developed breeds showed > 90% survival during the bioassay experiments under laboratory conditions.
- 5) A modified multiplex PCR was developed for the identification of major pathogens in *Bombyx mori*.

Utilization of the output

The Future line of work includes on station evaluation of *Bombyx mori* bidensovirus (*BmBDV*) resistant SK6R X SK7R silkworm hybrids at stations under CSRTI, Berhampore and On-Farm Trials for the evaluation of *BmBDV* resistant Double Hybrids FC1R X FC2R at farmer's level.

TRAINING

A 10 days training program entitled **Hands on Training on Molecular Techniques Applied in the Field of Sericulture** w.e.f. 14.11.2022 to 23.11.2022 was organized in which 22 participants including CSB Scientists/staff from CSRTI, Mysore & Berhampore, CSGRC, Hosur, SSTL, Kodathi and students from UAS, GKV Bengaluru, Oxford University, MGR College Hosur, Jain University, Bangalore were attended.



SILKWORM BREED 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 30th meeting of the Research Advisory Committee was held on 25th August, 2022 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.

Meeting / Workshop / Conference / Symposium / Seminar Attended

1. **Dr. Ramesha A, Dr. Pawan Shukla, Dr. K. S. Tulsi Naik, Dr. G. Subrahmanyam and Dr. Himanshu Dubey** attended the National Seminar on Climate Smart Sericulture 2022" Approaches for Sustainable Sericulture. Organized by RCS, CO, CSB Bangalore during October 6th – 7th, 2022, NIFT Bengaluru, India
2. **Dr. Rajal Debnath** attended physically while **Dr G. Subrahmanyam, Dr. K. S. Tulsi Naik & Dr. Himanshu Dubey** attended online and presented oral paper in the 26th International Sericulture Commission Congress, Seritech: New Concepts in Sericulture, 7th-11th September 2022, Cluj-Napoca, România.
3. **Dr. K. M. Ponnuvel, Dr. G. Subrahmanyam and Dr. Himanshu Dubey** participated in the National Symposium on Vanya sericulture-Opportunities Galore organized by CTR&TI, CSB Ranchi during 28th to 29th October 2022.
4. **Dr. Himanshu Dubey and Dr. Rajal Debnath** attended One-month International workshop on "Drug discovery and Development" through online mode from 15th May to 15th June 2022 organized by Decode Life
5. All the Scientists of this Institute organized and participated in Intellectual property rights conducted in association with Karnataka state council for science and technology on 23rd May 2022 at CSB Bangalore-560068

Abstracts Published in Workshops / Conference / Symposium / Seminar

1. K S Tulsi Naik, Vanitha C, G. Raghavender, A. Ramesha, Lakshmanan Velusamy, Mihir Rabha, M. S. Ranjini, Chandana., K M Ponnuvel, B.T. Sreenivasa, V.Sivaprasad, R. K. Mishra (2022) Molecular marker assisted breeding for the development of *BmBDV/BmDNV2* resistant productive silkworm breeds In 26th International Sericulture Commission Congress, Seritech: New Concepts in Sericulture, 7th-11th September, Cluj Napoca, Romania
2. G. Subrahmanyam, KM Ponnuvel, H. Dubey, V. Sivaprasad, A. Jagadish, D. Khajje (2022) Identification and characterization of the pathogen causing virosis in muga silkworm, *Antheraea assamensis*. Pp: 115. The 26th International Sericulture Commission Congress, Seritech: New Concepts in Sericulture, 7th -11th September 2022, Cluj-Napoca, România.
3. Indumathi Kamatchi. B, Himanshu Dubey, Kangayam. M. Ponnuvel, V. Sivaprasad, K. Sathyanarayana, J.P. Pandey and K.P. Arun Kumar (2022) Comparative analysis of whole mitochondrial genome of *Antheraea* silkworms (Lepidoptera: Saturniidae) In 26th International Sericulture Commission Congress, Seritech: New Concepts in Sericulture, 7th-11th September 2022, Cluj-Napoca, România.
4. Khajje D, Devi SS, Jagadish A, Dubey H, Subrahmanyam G, Kobayashi J, Terenius O, Ponnuvel KM. (2022) Molecular landscape of *A. proylei* nucleopolyhedrosis virus infecting Oak tasar sericulture, Pp: 138. The 26th International Sericulture Commission Congress, Seritech: New Concepts in Sericulture, 7th -11th September 2022, Cluj-Napoca, România.

5. G. Subrahmanyam, R. Das, R. Debnath, M. Chutia, H. Dubey, K. M. Ponnuvel (2022) Characterization of bacterial pathogens in muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: saturniidae). Pp:102. National Symposium on Vanya sericulture-Opportunities Galore organized by CTR&TI, CSB Ranchi during 28th to 29th October 2022.
6. Ramesha A, Naleen, Himanshu Dubey, Prashanth Sangannavar, Shreya Anthony, Raju Mondal, K.M. Ponnuvel, B.T. Srinivasa, V. Sivaprasad, R.K. Mishra, Tanmoy Sarkar. Doubled haploids: Opportunities and challenges in mulberry crop improvement. "National Seminar on Climate Smart sericulture -2022" 6th - 7th Oct. 2022. Oral presentation.
7. Pawan Shukla, Ramesha A. Reddy, Kangayam M. Ponnuvel (2022). Genome-Wide Characterization and Abiotic Stresses Expression Analysis of putative genes associated with low-temperature stress tolerance in mulberry varieties in National Seminar on "Climate Smart Sericulture -2022: Approaches for Sustainable Sericulture" held on 6th & 7th October, 2022 organized by Central Silk Board, Bangalore
8. G. Subrahmanyam, M. Kalita, D. Iyyangar, P. Sangannavar, H. Dubey, R. Debnath. K. M. Ponnuvel, BT Sreenivasa (2022). Characterization of a fungal pathogen causing muscardine disease in muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: saturniidae). Pp: 98, National Seminar on Climate Smart Sericulture 2022" Approaches for Sustainable Sericulture. Organized by RCS, CO, CSB Bangalore during October 6th – 7th, 2022, NIFT Bengaluru, India.
9. Borah LM, Subrahmanyam G, Mahesh DS, Arunkumar KP. (2022) Efficacy of chemical agents against pebrine disease of Muga silkworm, *Antheraea assamensis*. Pp.103. National Seminar on Climate Smart Sericulture 2022" Approaches for Sustainable Sericulture. Organized by RCS, CO, CSB Bangalore during October 6th – 7th, 2022, NIFT Bengaluru, India.
10. K S Tulsi Naik, Vanitha C, G. Raghavender, A. Ramesha, Lakshmanan Velusamy., Mihir Rabha, M. S. Ranjini, Chandana., K M Ponnuvel, B.T. Sreenivasa, V.Sivaprasad, R. K. Mishra (2022) Molecular marker-assisted breeding and development of *Bombyx mori* bidensovirus (BmBDV)resistant thermo tolerant silkworm breeds and hybrids at the National seminar on Climate Smart Sericulture 2022 approaches for sustainable sericulture, organized by Central Silk Board at National Institute of Fashion Technology, Bangalore
11. Ponnuvel KM, Khajje D, Jagadish A, Subrahmanyam G, Bhatt S. (2022) Lateral flow assay kit (LFA) for the early detection of pebrine in silkworms. Pp: 96. National Symposium on Vanya sericulture-Opportunities Galore organized by CTR&TI, CSB Ranchi during 28th to 29th October 2022.
12. Singh S, Subrahmanyam G, Sangannavar P, Kumar R, Choudhury B, Jigyasu DK, Luikham R, Kumari VJ. (2022) Conservation of Muag silkworm, *Antheraea assamensis* Helfer in different geographical locations of North East India. Pp: 99. National Symposium on Vanya sericulture-Opportunities Galore organized by CTR&TI, CSB Ranchi during 28th to 29th October 2022.
13. Himanshu Dubey, Aneesha P.J., Suraj Kumar Shah, K. M. Ponnuvel, and K.P. Arunkumar (2022) Identification of circadian rhythm related genes in *Antheraea assamensis* (Helfer) through In-silico approach in National Symposium on Vanya sericulture-Opportunities Galore organized by CTR&TI, CSB Ranchi during 28th to 29th October 2022.
14. Pawan Shukla, and Kangayam M. Ponnuvel, (2023). जैव प्रौद्योगिकी तकनीकी के माध्यम से शहतूत पौध का उन्नयन published in राष्ट्रीय राजभाषा तकनीकी सेमिनार organized by CTRTI, Ranchi on 28.01.2023 ISBN No. 978-81-958770-1-0

राजभाषा कार्यान्वयन - OFFICIAL LANGUAGE IMPLEMENTATION

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

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

- संस्थान ने अनुच्छेद 3(3) के प्रावधानों का अनुपालन करते हुए सभी दस्तावेज द्विभाषी में जारी किए गए।
The Institute has followed the provisions of article 3(3) and issued all documents in bilingual.
- संबंधित क्षेत्रों में हिंदी में पत्राचार के लक्ष्य (जोन ए में 100%, जोन बी में 100% और जोन सी में 55%) प्राप्त किए गए थे। 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 2023 में SSTL और RSRS, के साथ मिलकर एक दिवसीय संयुक्त कार्यशाला का आयोजन किया गया।
One joint workshop was organized in March 2023 jointly with SSTL and RSRS, Kodathi.
- हिंदी पखवाड़ा 14 से 30 सितंबर 2022 तक मनाया गया और विभिन्न कार्यक्रम आयोजित किए गए।
Hindi Pakhwada was celebrated from 14th-30th September 2022 by conducting various events.

PUBLICATIONS

1. Channabasava Veershetty, Harshitha Prakash, Pawan Shukla, and Kangayam M. Ponnuvel (2023) Study on the anatomy of the pink mealybug *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) using stereo microscopy. International Journal of Tropical Insect Science, 43, 861–867.
2. Juthika Saikia, Rhitu Kotoky, Rajal Debnath, Niraj Kumar, Priyanka Gogoi, Archana Yadav, Ratul Saikia, (2023) De novo genomic analysis of *Enterobacter asburiae* EBRJ12, a plant growth-promoting rhizobacteria isolated from the rhizosphere of *Phaseolus vulgaris* L, Journal of Applied Microbiology, 134(2), 1xac090
3. G. Subrahmanyam, R. Das. R. Debnath, M. Chutia, H. Dubey, KM. Ponnuvel, K. (2023) Characterization of bacterial pathogens in muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: saturniidae). Journal of Environmental Biology, 44, 479-48
4. Tulsi Naik K S., Ismail S., Pradeep A.R. et al. (2023). 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. Appl Biochem Biotechnol Springer, 195, 2371–2394
5. Esvaran V. G., Ponnuvel S., Jagadish A., Savithri H. S., Subramanya H. S., & Ponnuvel K. M. (2022). Cloning, Expression and Characterization of Spore Wall Protein 5 (SWP5) of Indian Isolate NIK-1S of *Nosema bombycis*. The Protein Journal, 41(6), 596-612.
6. Khajje D., Devi S. S., Subrahmanyam G., Kobayashi J., Sivaprasad V., Terenius O., & Ponnuvel K. M. (2022). Investigation on Pathological Aspects, Mode of Transmission, and Tissue Tropism of *Antheraea proylei* Nucleopolyhedrovirus Infecting Oak Tasar Silkworm. Journal of Insect Science, 22(5), 9.
7. Sandilya S. P., Jeevan B., Subrahmanyam G., Dutta K., Vijay N., Bhattacharyya N., & Chutia M. (2022). Co-inoculation of native multi-trait plant growth promoting rhizobacteria promotes plant growth and suppresses Alternaria blight disease in castor (*Ricinus communis* L.). Heliyon, 8(12), e11886.
8. Singh S., Jigyasu D. K., Subrahmanyam G., Sangannavar P., Kumar R., Choudhury B. N., & Vijayakumari K. M. conservation of muga silkworm, *Antheraea assamensis* Helfer in the natural habitats at different geographical location. Plant Archives 22, 230-239.
9. Kangayam M. Ponnuvel, Tulsi Naik K. S., Ramesha A. Reddy, Pawan Shukla, Subrahmanyam G., Rajal Debnath, and Himanshu Dubey (2022) Status paper on Seri-Biotechnology in mulberry varieties in

National Conference on National Seminar on 'Climate Smart Sericulture - 2022: Approaches for sustainable sericulture organized by Central Silk Board on 6th -7th October, 2022

10. Kalita M., Chutia M., Jha D.K. and Subrahmanyam G. (2022) Mechanistic understanding of *Gordonia* sp. in biodesulfurization of organosulfur compounds. *Current Microbiology*, 79(3), p.82.

Books/Book chapters

1. Chitara M.K., Sharma S., Parihar M., Jeevan B., Sangannavar P.A., Kumar A., & Subrahmanyam G. (2022). The structure, function, and utility of the rhizosphere microbiome of cereal crops. In *Microbial Resource Technologies for Sustainable Development* (pp. 77-111). Elsevier publisher
2. Sangannavar P.A., Kalita M., Subrahmanyam G., Kumar, A., Sathyanarayana, K., & Debnath, R. (2022). Biomethane: a sustainable bioenergy source from potential waste effluents. In *Microbial Resource Technologies for Sustainable Development* (pp. 195-212). ISBN: 978-0-323-90590-9 Elsevier publisher
3. Kalita M., Sangannavar P.A., Chutia M., Jha D.K., Sathyanarayana K., Kumar J.S., & Subrahmanyam G. (2022). Microbial biodesulfurization: a sustainable technology for refining fossil fuels. In *Microbial Resource Technologies for Sustainable Development* (pp. 333-351). ISBN: 978-0-323-90590-9 Elsevier publisher.

AWARDS

1. **Dr. K. M. Ponnuvel**, Scientist D and **G. Subrahmanyam**, Scientist C received the **best oral presentation award** in the National Symposium on Vanya sericulture-Opportunities Galore organized by CTR&TI, CSB Ranchi during 28th to 29th October 2022.
2. **Dr. G. Subrahmanyam**, Scientist C received the **best oral presentation award** in the National Seminar on Climate Smart Sericulture 2022" Approaches for Sustainable Sericulture. Organized by RCS, CO, CSB Bangalore during 6th – 7th October 2022, NIFT Bengaluru, India.
3. **Dr. K.S. Tulsi Naik**, Scientist C received **best oral presentation award** in the National Seminar on Climate Smart Sericulture 2022" Approaches for Sustainable Sericulture. Organized by RCS, CO, CSB Bangalore during 6th – 7th October 2022, NIFT Bengaluru, India.

FOREIGN VISITS

1. Dr. K. M. Ponnuvel, Scientist D visited Uppsala University, Sweden as part of Indo-Swedish Project Collaboration from 06th -11th September 2022
2. Dr. Rajal Debnath, Scientist C visited Cluj Napoca, Romania to attend the 26th international sericultural commission congress" organized by University of Agricultural sciences and veterinary medicine from 07th -11th September 2022



Silkworm breeds submitted to CSGRC Hosur for registration

BmBDV resistant lines

1. CSR26BR 2. CSR6BR 3. SK6BR 4. SK7BR

RESEARCH ADVISORY COMMITTEE

SN	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 Institute of Bioinformatics and Applied Biotechnology Bangalore	Member
3	Dr. Mohan Principal Scientist Division of Entomology, NBAIR, Bangalore	Member
4	Dr. Nataraj Karaba Professor, Dept. of Crop Physiology, UAS, GKVK, Bangalore	Member
5	Dr. K. Vijayan, Retired Scientist-D, Central Silk Board	Member
6	The Director CSR&TI, Mysore	Member
7	The Director CSGRC, Hosur	Member
8	The Director Seri-Biotech Research Laboratory Kodathi, Carmelram post, Sarjapur Road Bangalore	Member- Convener

INSTITUTE BIO SAFETY COMMITTEE

(Since Dec. 2020)

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

HUMAN RESOURCES

SN	Name	Designation
1	Dr. V. Sivaprasad	Director (Upto 31.05.2022)
2	Dr. K. M. Ponnuvel	Scientist- D & Officer In-charge (From 01.06.2022)
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- C
9	Mr. R. N. Srikantaiah	Senior Technical Assistant
8	Mr. K. M. Humayun	Senior Technical Assistant
9	Mr. G. Raghavendar	Field Assistant
10	Mr. Srinivas Raju K.V.	Field Assistant

Research fellows / Assistants

SN	Name	Designation
1.	Ms. Anupama Jagadish	Ph.D. Scholar & Senior Research Fellow
2	Ms. Deeksha Khajje	Ph.D. Scholar & Junior Research Fellow
3	Ms. Aneesha P.J.	Junior Research Fellow
4	Ms. Indumathi Kamatchi	Ph.D. Scholar & Junior Research Fellow
5	Ms. Vanitha C.	Ph.D. Scholar & Project Assistant
6	Ms. Arathi	Junior Research Fellow
7	Mr. Naleen	Junior Research Fellow
8	Mr. Channabasava Veershetty	Junior Research Fellow
9	Ms. L. Deepika Iyyangar	Junior Research Fellow
10	Ms. Lavanya C.	Junior Research Fellow
11	Ms. Harshitha Prakash	Junior Research Fellow

Administration

SN	Name	Designation
1	Ms. Manjula S.	Superintendent
2	Shri Kenchappa	Multi tasking Staff

SUPERANNUATION



Dr. V. Sivaprasad, Director; and Shri K. M. Humayun, STA retired from service of Central Silk Board, MoT, Gol 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

Hindi Fortnight

The Hindi fortnight was observed from September 14th-30th September 2022 including Hindi Day and many competitions were organized on the occasion of Hindi fortnight celebrations. During the fortnight, **Silk Day** was also observed on 20th September 2022 to pay homage to the visionary Dr. Syama Prasad Mookerjee and his pivotal role in shaping India's silk industry.

National Unity Day

The National Unity Day was observed on 31st October 2022 and **Run for Unity** was organized in which staff of SBRL, SSTL and RSRS, Kodathi have actively participated.

Intellectual Property Rights Workshop

One-day workshop was organized on “Intellectual property rights” in association with Karnataka State Council for Science & Technology, IISC, Bangalore on 23.05.2022.

Invited Talk

Prof. Ramanjulu Sunkar, Professor, Oklahoma State University, Oklahoma was invited to deliver a talk on “Role of miRNA in Plants” on 9th May 2022.

Yoga Day

Yoga day was organized on 21st June 2022 in which all the staff of SBRL were actively participated





रेशम जैव-प्रौद्योगिकी अनुसंधान प्रयोगशाला SERI-BIOTECH RESEARCH LABORATORY

Central Silk Board
Ministry of Textiles, Govt. of India
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