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2020 - 2021

रेशम जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला
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Annual Report 2020-21

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COVER PAGE

On the right side of the cover page shows the photographs of cocoons from different silkworms reared all over India.

Toward the left side of the cover page, the top most image is the photograph of callus induction from anther in V1 variety. The middle photo illustrates the Trasmission Electron Microscopy image of occlusion bodies of the *Anpr*NPV derived from infected *A. proylei* silkworms. The third or last is the gel photographs of semi-quantitative PCR showing the vertical transmission of *A. mylitta* Iflavirus in infected *A. proylei* gravid females.

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प्राक्कथन

मेरे लिए वर्ष 2020-21 की वार्षिक रिपोर्ट प्रस्तुत करना सौभाग्य की बात है जिसमें हमने रेशम जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला के विजन और मिशन के साथ अपने लक्ष्यों को संरेखित करते हुए विभिन्न महत्वपूर्ण उपलब्धियां हासिल की है । इस महामारी के सबसे कठिन समय के बावजूद, हमारे वैज्ञानिकों और कर्मचारियों ने अपना सर्वश्रेष्ठ प्रदर्शन करने के लिए अथक कार्यशैली का प्रदर्शन किया है, जिसके लिए वे सराहना के पात्र हैं ।

इस वित्त वर्ष में प्रयोगशाला ने आठ परियोजनाओं का क्रियान्वयन जारी रखा जिनमें दो परियोजनाएं सम्पूर्ण हुई । इस वर्ष के दौरान विभिन्न उपलब्धियों में पश्चिम बंगाल और हिमाचल प्रदेश में प्रचलित संकरों की तुलना में न्यूक्लियोपॉलीहेड्रो वायरस (एनपीवी) संक्रमण से प्रतिरोध / सहिष्णुता के लिए लक्षित नए विकसित संकर (एमएएसएन4 x सीएसआर4) के लाभों का पता लगाना; *एंथेरिया प्रोयली* में टाइगर बैंड रोग के लिए अंडे की सतह से वायरल संदूषण के संचरण की स्थापना करने के साथ-साथ अंडे की सतह के लिए कीटाणुशोधन तकनीक का विकास करना तथा इफ्लावायरस जो कि *ए. मायलिट्टा* में वायरोसिस रोग पैदा करने के लिए एक नए रोगज़नक के रूप में जिम्मेदार है, का जीनोमिक डेटा का विश्लेषण महत्वपूर्ण थे। इस इफ्लवायरस के जीनोम डेटा से पता चला है कि यह *ए. पर्नी* इफ्लावायरस से निकटता रखता है। चूर्णिल आसिता रोग प्रतिरोधी शहतूत के विकास के लिए, दो मिल्ड्यू रेसिस्टेंस लोकस ओ (एमएलओ) जीन, एमएलओ2 और एमएलओ6 ए को रोग के संवेदनशीलता से जुड़े होने के रूप में पहचाना गया है ।

केन्द्रीय रेशम बोर्ड के सदस्य सचिव और निदेशक (वित्त) के समर्थन और मार्गदर्शन के बिना उपरोक्त अनुसंधान गतिविधियां संभव नहीं थी। शोध प्रस्ताव तैयार करने से लेकर और पूर्ण होने तक, अनुसंधान सलाहकार समिति से अपार समर्थन मिला है। हमारी वैज्ञानिक बिरादरी और बुनियादी ढांचे में विभिन्न एजेंसियों - डीबीटी/डीएसटी / स्वीडिश अनुसंधान परिषद द्वारा दिये गए अनुसंधान समर्थन और विश्वास के लिए धन्यवाद ज्ञापित करते है । सहयोगी भागीदारों के योगदान के लिए भी आभार व्यक्त करते है ।

मुझे विश्वास है कि पाठकों को यह संस्करण बहुत उपयोगी और ज्ञानवर्धक लगेगी ।

डॉ. आर. के. मिश्रा
निदेशक

FOREWORD

It is my privilege to present the Annual Report of Seri-Biotech Research Laboratory, Central Silk Board, Kodathi for the year 2020-21 during which we have achieved various important milestones aligning our goals with the vision and mission. In spite of the toughest times amidst this pandemic, our scientists and staff have relentlessly performed to deliver their best, for which they deserve appreciation.

The laboratory continued with implementation of eight projects and a total of two were concluded during this financial year. Among different achievements during the year, ascertaining the benefits of the newly developed hybrid (MASN4 x CSR4) targeted to tolerate nucleopolyhedro virus (NPV) infection, against the ruling hybrids in West Bengal and Himachal Pradesh; establishing egg surface transmission of viral contamination of the Tiger band disease in *Antheraea proylei* together with development of egg-surface disinfection technique and addition of genomic data on the Iflavirus, a new pathogen causing virosis disease in *A. mylitta* were notable findings. The genomic details of the iflavirus deciphered it to be closely related to *A. pernyi* Iflavirus. Towards development of mildew resistant mulberry, two Mildew Resistance Locus O (MLO) genes, MLO2 and MLO6A have been identified as linked to susceptibility to the disease.

The aforesaid research activities were not possible without the support and guidance from the Member Secretary and Director (Finance) of Central Silk Board. The support from Research Advisory Committee has been immense, right from drafting research proposals to their execution and completion. The research support and confidence shown by different agencies- DBT/ DST/Swedish Research Council in our scientific fraternity and infrastructure is acknowledged. The contributions by collaborating partners are equally acknowledged.

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

Dr. R. K. Mishra
Director

VISION AND MISSION

VISION

To become a Centre of Excellence in Seribiotechnology.

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 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
- 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 seribiotechnology

अनुसंधान के मुख्य आकर्षण

1. एनपीवी प्रतिरोध / सहिष्णुता के लिए एमएएसएन से विकसित द्विप्रज संकर, एमएएसएन4 x सीएसआर4 ने पश्चिम बंगाल और हिमाचल प्रदेश में विद्यमान संकरों की तुलना में बेहतर उपज और कोकून गुणवत्ता दिखाई है ।

2. एसएएसएन लाइन से विकसित क्रॉसब्रीड, निस्तारी x एमएएसएन4 ने भी उपज और कोकून की गुणवत्ता में पश्चिम बंगाल में विद्यमान संकरों की तुलना में बेहतर प्रदर्शन किया है ।

3. *एंथीरिया प्रॉयली* में टाईगर बैंड रोग के लिए जिम्मेदार रोगजनक वायरस, *एंथीरिया प्रॉयली* के अंडे की सतह के साथ-साथ नवजनित रेशमकीट में पाया गया है जो कि दर्शाता है कि अंडे संक्रमण का संभावित स्त्रोत है ।

4. *एंथेरिया प्रोयली* के अंडे की सतह को 0.2% सोडियम हाइपोक्लोराईट (NaOCl) कीटाणुनाशक से विसंक्रामित करके *एंथेरिया प्रोयली* में टाइगर बैंड रोग को प्रभावी तरीके से नियंत्रित किया जा सकता है ।

5. जीनोम अनुक्रमण के माध्यम से *एंथेरिया मायलिट्टा* को संक्रमित करने वाले इफ्लावायरस के जीनोम का संपूर्ण विश्लेषण किया गया । सह - संक्रमण अध्ययनों से जानकारी प्राप्त हुई कि *एंथेरिया मायलिट्टा* में नोसेमा इफ्लावायरस से जुड़ा एक प्रमुख रोगजनक है जबकि *एंथेरिया प्रोयली* में एएनपीआरएनपीवी एक प्रमुख रोगज़नक है ।

6. विभिन्न उतकों जैसे वसा शरीर, मध्यांत्र, मैलपीगी नलिका तथा अंडाशय में विषाणु के प्रगुणन का पता लगाया गया और मातृ शलभ से संतति में विषाणु के संचरण की भी पुष्टि की गई है।

7. शहतूत में चूर्णिल आसिता रोग संवेदनशीलता में शामिल दो मिल्ड्यू रेसिस्टेंस लोकस ओ (एमएलओ) जीन, एमएलओ 2 और एमएलओ 6 ए की उम्मीदवार जीन के रूप में पहचान की गई है ।

8. अगुणित (हैप्लोइड) विकास के लिए शहतूत में एथेर कल्चर से माइक्रोस्पोर्स के कोशिका विभाजन के संबंध में प्रारंभिक प्रतिक्रिया के लिए ऊतक संवर्धन परिस्थितियों की पहचान की गई है ।

9. बाइडेन्सोवायरसप्रतिरोध जीन रखने वाली छह द्विप्रज नस्लों की पहचान की गई साथ ही एसके-6 और एसके-7 नस्लों को प्रतिरोध जीन के लिए समयुग्मजी के रूप में विकसित किया गया है ।

10. बायोएॅसे ने पुष्टि की कि जब पेब्राइन बीजाणु संक्रमित होते हैं और इसके अंर्तनिंहित आनुवंशिक तंत्र की जांच की जा रही है, तो लैमेरिन नस्ल बहुत अधिक जीवितता दिखाती है ।

RESEARCH HIGHLIGHTS

1. The bivoltine hybrid developed from MASN line, MASN4 x CSR4 for NPV tolerance showed better cocoon yield and quality than ruling hybrids in West Bengal and Himachal Pradesh

2. The crossbreed developed from MASN line, Nistari x MASN4 performed better than the existing hybrids for cocoon yield and quality in West Bengal.

3. The virus pathogen responsible for Tiger band disease in *Antheraea proylei* was detected on the surface of the eggs as well as in the freshly hatched silkworms indicating eggs as a potential source of infection.

4. Surface sterilization of eggs with 0.2 % sodium hypochlorite (NaOCl) disinfectant was effective in controlling Tiger band disease in *Antheraea proylei*

5. The genome of Iflavirus infecting *Antheraea mylitta* was characterized through genome sequence and co-infection studies revealed Iflavirus as a major pathogen associated with *Antheraea mylitta* whereas, AnprNPV was a major pathogen in *Antheraea proylei*.

6. The multiplication of Iflavirus in *Antheraea mylitta* was detected in various tissues such as fat body, midgut, malpighian tubule and ovary. Vertical transmission was also confirmed from mother moth to the offspring.

7. Identified two Mildew Resistance Locus O (MLO) genes, MLO2 and MLO6A as candidate genes involved in powdery mildew disease susceptibility in mulberry

8. Tissue culture conditions were standardized for initial response with respect to cell division of microspores from anther culture in mulberry for development of haploids

9. Identified six bivoltine breeds possessing Bidensovirus resistance gene and developed SK6 and SK7 breeds homozygous for the said resistance gene

10. Bioassay validated that Lamerin breed show very high survivability on infection by pebrine spores.

LIST OF RESEARCH PROJECTS

ON-GOING RESEARCH PROJECTS

1. **ARP-08001CI** : Studies on the genetic characterization, transmission and tissue distribution of Iflavirus infecting the Indian tropical tasar silkworm, *Antheraea mylitta* (April 2018 – March 2022), **Indo-Swedish Funded**

2. **PRP-08002MI** : Identification of powdery mildew resistant genes and validation of CAPS marker for Chalcone synthase in mulberry spp. (May 2019- May 2022)

3. **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**

4. **PIT-08004MI** : Study on epigenetic and autophagy modifiers on induction of haploid microspore embryogenesis in mulberry (March 2020- February 2023)

5. **AIT-08005MI** : Development and evaluation of Bidensovirus resistant silkworm hybrids developed from marker assisted breeding lines -Phase II (March 2020-February 2023)

6. **AIT-08006 EF** : Development of lateral flow assay (LFA) kit for diagnosis of pebrine disease in silkworms (March 2021 - January 2023), **DBT-BIRAC Funded**

CONCLUDED RESEARCH PROJECT

1. **ARP-3605** : Validation of the DNA markers in silkworm breed developed by introgression of DNA markers associated with NPV resistance using Marker Assisted Selection Breeding and large scale field trial of the breed (February 2017 – August 2020), **DBT Funded**

2. **ARP-3606** : Development of diagnostic tool for early detection of baculovirus causing tiger band disease in *Antheraea proylei* (February 2017 – August 2020), **DBT Funded**

PROGRESS OF ON-GOING RESEARCH PROJECTS

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

(Funded by Indo-Swedish collaboration; **Duration:** April 2018 – March 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

Iflavirus causes virosis in *A. mylitta* silkworms, which are indigenous to India and are wild silkworms reared outdoors (**Fig. 1**). The Iflavirus infecting these silkworms is named as "*A. mylitta* Iflavirus" (*Am*IV) based on its molecular characterisation. The genome of the novel Iflavirus has been sequenced and can be found under the *accession no. MW115117.2* on NCBI. The genome organisation reveals that the Iflavirus is a positive strand 9728 nucleotides long RNA virus. The genome codes for a 2967 amino acid long polyprotein which further undergoes autocatalytic cleavage into individual proteins. The structural capsid proteins in the order VP2-VP4-VP3-VP1 are found at the 5' end and non structural proteins in the order *Helicase, 3C protease, RdRp*- AAA 3 at the 3' end (**Fig. 2**). The genome has poly A tail at the 3' end and a short leader sequence at the 5' end. Phylogenetic analysis of the polyprotein revealed that, the *Am*IV is closely related to *Ap*IV, chinese oak tasar with a 91% similarity of amino acids between these species (**Fig. 3**).

The identified Iflavirus causes systemic infection spreading across various tissues such as epidermis, midgut, fat body and ovary. The virus transmits vertically from the infected female moths to the offsprings and propogates well in the host in presence of co-infection of other virus such as *Anpr*NPV, spreading through different development stages as compared to NPV **(Fig. 4).**

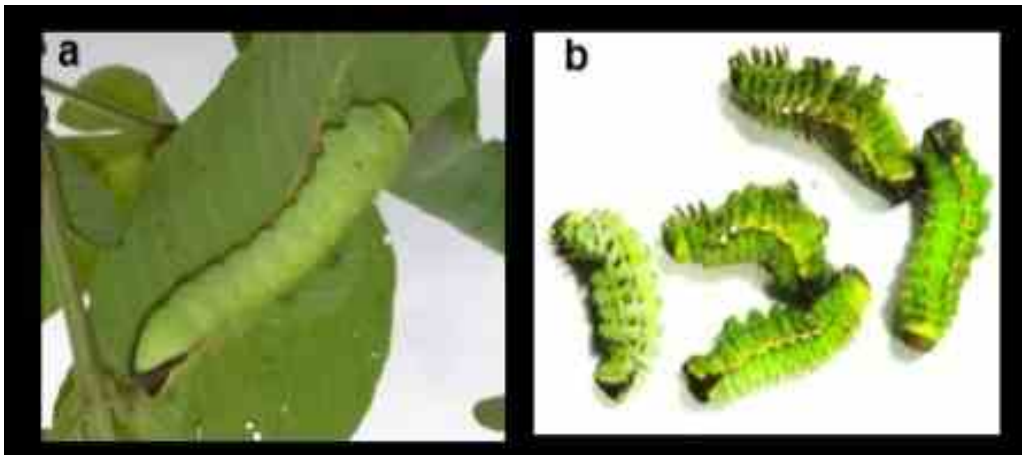

Figure 1: *Antheraea mylitta* larvae. a: healthy larva, b: infected larvae

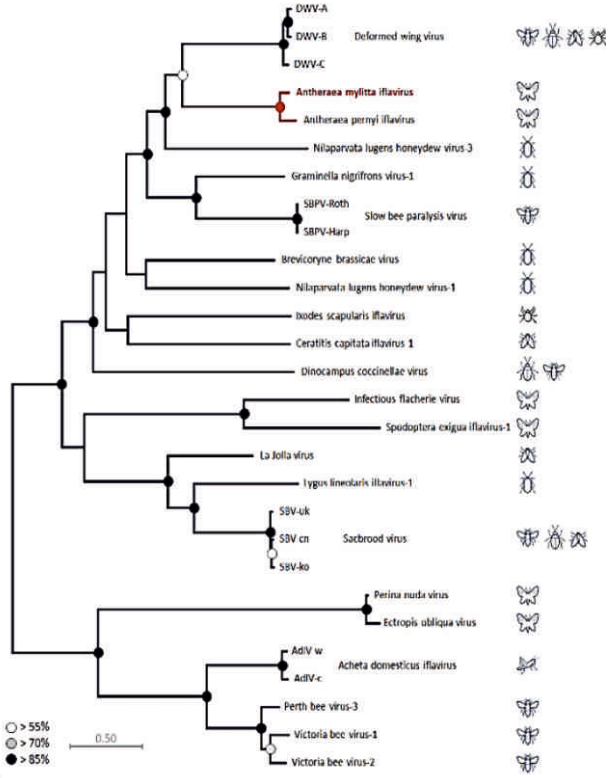

Figure 3 : Phylogenetic analsis of the AmIV with other picornavirales family of Iflavirus

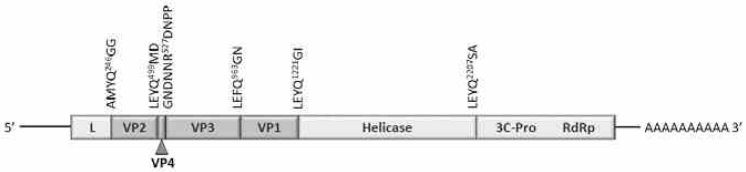

Figure 2: Genome organisation of *A. mylitta* Iflavirus. L: Leader peptide, VP2, VP4, VP3, VP1- Viral Proteins, 3C Pro: 3C Protease, RdRp: RNA Dependent RNA polymerase

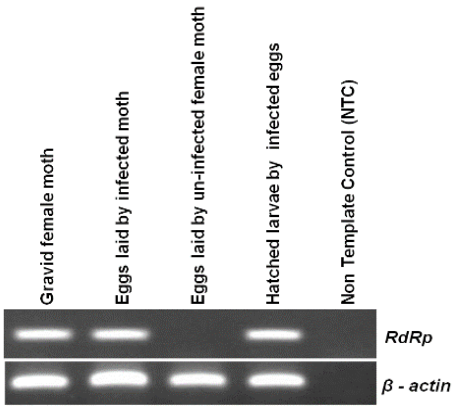

Figure 4: Vertical transmission observed for AmIV in infected *A. proylei* gravid females

The transfer through vertical route is indicative of greater virulence. The *Am*IV hence identified in this study can be termed as virulent strain of Iflavirus.

Currently development of disease diagnostic kit is being attempted based on the VP proteins that have been identified from the AmIV genome. The cDNA samples isolated from the infected *A. mylitta* silkworms have been used to amplify the viral proteins. The amplified product has been cloned into expression vector for expression of viral capsid proteins in the bacterial expression system.

2. PRP-08002MI: 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* and K. Vijayan# (upto 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 disease negatively affects quality and quantity of mulberry leaf available to produce silk. Genetic resistance through development of resistant / tolerant varieties forms an important approach for management of the disease. Identification of Susceptibility (S) genes and substitute Resistance (R) genes is essential for disease resistance breeding so as to confer broad spectrum and durable resistance. Mildew resistance Locus O (MLO) based resistance is successfully being utilized in commercial plant breeding in few crops to counter powdery mildew fungi infection.

In this project, genome wide analysis identified 16 MLO genes in mulberry. Phylogenetic analysis revealed that, MnMLO2, MnMLO6A, MnMLO6B, MnMLO12A and MnMLO12B clustered with functionally characterized MLOs associated with powdery mildew susceptibility in dicot species. Gene expression analysis indicated increased transcript abundance of MnMLO2, MnMLO6A, and MnMLO12A in response to powdery mildew infection. Further Mildew Resistance Locus O (MLO) proteins were analyzed for conserved motifs associated with powdery mildew susceptibility using MEME suite. Parameters were adjusted to identify five conserved motifs at the C-terminus with default motif width. Figure (**Fig. 5**) shows that Motif 1 is common to all the MLO proteins analyzed and found just downstream of last transmembrane domain. Motif 2 and Motif 5 were present in all the functionally characterized MLO proteins involved in powdery mildew susceptibility from other plant species, whereas, in *M. notabilis* these motifs were present in only two (MnMLO2 and MnMLO6A) out of total five clade V MnMLOs. Interestingly these motifs were also present outside clade V MnMLOs (MnMLO1C contains motif 2 and motif 5, whereas, MnMLO8 contains only motif 5).

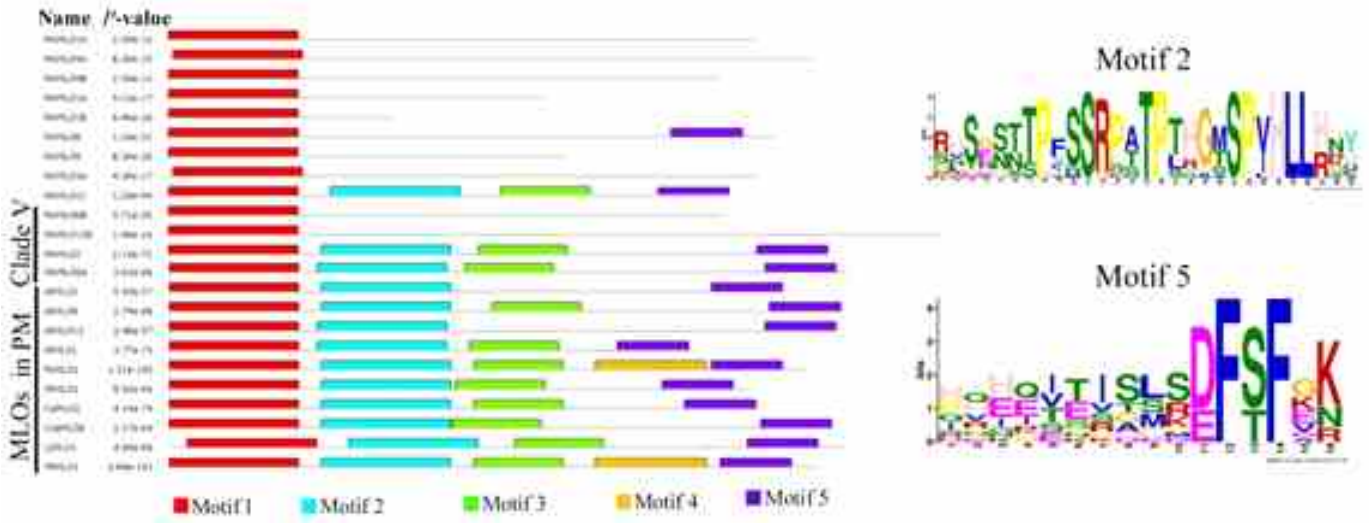

Figure 5: Schematic representation of conserved motifs in MLO proteins. Conserved motifs of MLO proteins from *Morus notabilis* and characterized MLO proteins from different dicot plants using MEME suite. Sequence logo of Motif 2 and Motif 5 are shown and are specific to all the characterized MLO proteins and for MnMLO1C, MnMLO2 and MnMLO6A proteins.

Thirty amino acids have been described as invariable for the MLO proteins. We looked for the conservation of these invariable amino acids in MnMLO proteins. Majority of MnMLOs (MnMLO1A, MnMLO1B, MnMLO1C, MnMLO2, MnMLO4A, MnMLO6A, MnMLO8, MnMLO12A, MnMLO13 and MnMLO14) contain all the 30 invariable amino acids, while few changes have been observed in some of the MnMLOs.

In summary, genome wide analysis identified 16 MLO genes from the *M. notabilis* genome. The identified proteins have characteristic domains of MLO proteins such as MLO, transmembrane and calmodulin binding domains. Phylogenetic analysis identified MnMLO2, MnMLO6A, MnMLO6B, MnMLO12A and MnMLO12B as clade V genes. Further, conserved motifs exclusively present in functionally characterised MLOs involved in powdery mildew susceptibility were present in MnMLO1C, MnMLO2 and MnMLO6A. Gene expression analysis identified MnMLO2, MnMLO6A and MnMLO12A induced in response to powdery mildew fungi inoculation. Combined analysis of phylogenetic tree, conserved motif analysis and gene expression identified MnMLO2 and MnMLO6A as potential candidate genes involved in powdery mildew susceptibility in mulberry.

3. AIT-08003CN: 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 - July 2022)

Tulsi Naik K. S., Upendra Nongthomba* and K. Vijayan# (upto May 2021)

*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 silkworm, *B. mori* and other related species are susceptible to different kinds of diseases caused by pathogens. The most common pathogens infecting them are microsporidians including *Nosema bombycis*, nucleopolyhedrovirus (NPV), densovirus, infectious flacherie virus (IFV), cytoplasmic polyhedrovirus (CPV), and bacteria. Among all pathogens, the microsporidia causing "**Pebrine disease"** is responsible for significant economic loss to the sericulture industry.

Among numerous silkworm, *B. mori* breeds, most are susceptible to microsporidian infections. The commercially productive CSR2 breed is highly susceptible to microsporidian infections, while, the silkworm breed which exhibited most resistance / tolerance to microsporidians is "**Lamerin" or "Leimaren**", belonging to Manipur, North East India where it is commercially exploited. It is also the only *B. mori* breed which survives with microsporidia infections. Preliminary work carried out at SBRL showed that, 35% of the Lamerin breed was resistant to microsporidia and 65% was tolerant. The reason as to what makes Lamerin resistant / tolerant to microsporidia unlike other bivoltine and multivoltine silkworm breeds is not known. An understanding of the comparative transcriptome is essential for interpreting the functional elements of the genome with respect to resistance / tolerance to pathogens (Wang *et al.,* 2009).

Rearing of Silkworms for Bioassay experiments

Lamerin, a resistant / tolerant breed to microsporidia was procured from CSB Research Extension Centre, Mantripukhri, Imphal, Manipur and reared at SBRL. The CSR2 breed, a commercial race showed better pre-cocoon characters due to the selection pressure on the breed and continuous domestication, whereas, Lamerin breed showed comparatively low pre-cocoon characters (**Fig. 6a & 6b**) (**Table 1**). Though the breeds show variations in the quality parameters,

as far as disease resistance is concerned, Lamerin breed shows better resistance to pathogens while the latter is more susceptible to pathogens.

a)  b) 

Figure 6: **(a)** Lamerin cocoons maintained at SBRL procured from CSB REC, Mantripukhri, Imphal, Manipur

(b) CSR2 commercial breed highly susceptible to microsporidia available at SBRL.

Table 1: Pre- cocoon characters of CSR 2 and Lamerin breeds

Race name	Type of race	Country	Egg shape	Larval body colour	Larval pattern	Cocoon colour	Cocoon shape
CSR-2	Evolved	India	Ellipsoidal	Bluish white	Plain	White	Oval
Lamerin	E(BM)	India	Ellipsoidal	Creamish white	Plain	Golden yellow	SB

Race name	Fecundity (no.)	Hatching %	Wt. of 10 grown larvae (g)	Pupation rate (%)	Single cocoon wt. (g)	Single shell wt. (g)	Silk ratio (%)
CSR-2	484.00	86.90	35.34	89.08	1.66	0.38	22.97
Lamerin	322.00	90.05	19.64	81.44	0.94	0.13	13.51

Microsporidian infection and differential gene expression analysis of host response genes

The third instar larvae (Day 1) of both breeds were starved for six hours and then fed with 5000 spores / larva of highly virulent microsporidian, *Nosema bombycis* smeared on mulberry leaves. The control groups were treated with sterile water. After infection, the larvae are reared on normal mulberry leaves. After 24h, 48h post infection, larval midguts (10 Nos) were dissected out, washed in DEPC treated water, suspended in Trizol reagent (Invitrogen) and kept at -80 ^{o}C. The

total RNA was extracted using Qiagen RNA extraction kits according to the manufacturer's protocol. The purity of all RNA samples was assessed at an absorbance ratio of A260/280 and A260/230 and the integrity of the RNA was confirmed through 1% agarose gel electrophoresis. The cDNA was synthesized using standard protocol. 1µl of cDNA was used for qPCR analysis.

Real-time quantitative PCR (qPCR) analysis

In order to validate the results from the mRNA the relative expression levels of selected genes that might be involved in microsporidia resistance was done by reverse transcription quantitative PCR (RT-qPCR) with SYBR green (**Table 2**). The total RNAs from midgut of both strains was used for cDNA synthesis and PCR. The relative quantification using comparative "Ct method" was used. The independent experiments were performed in triplicate.

Table 2: Silkworm host response genes that are upregulated at 24 - 48 hpi

Gene ID/Probe ID	Name	Reference
BGIBMGA013866 *	Gloverin 4-like (GL4)	Ma *et al.*, 2013
BGIBMGA014427-PA	Serine protease (SP1)	Li et al., 2018
BGIBMGA000017 *	Cecropin D1(CecD1)	Ma et al., 2013
sw09761 *	Moricin like-C2	Ma et al., 2013
BGIBMGA014429-PA	Serine protease	Li et al., 2018
BGIBMGA014431-PA	Serine protease	Li et al., 2018
sw09761 *	Moricin like-C2	Ma et al., 2013

Differential gene expression analysis of host response genes in CSR2 and Lamerin breeds

The gene expression patterns in the midgut of *CSR 2 (susceptible) and Lamerin (tolerant/ resistant)* infected with the microsporidian *N. bombysis* were investigated through qPCR. The qPCR analysis showed antimicrobial proteins, ie., gloverins, cecropins and moricins specifically expressed due to *N. bombycis* infection were significantly upregulated during 24 and 48 hpi indicating activation of the silkworm systemic immune response. The serine protease (Serpins) genes *SP1, SP3, SP4* were also significantly upregulated during 24 - 48 hpi. These Serpins are involved in elimination of pathogens and expression of serine proteases (SPs) as well as their activation is tightly regulated by serine protease inhibitors (serpins) the largest known superfamily of protease inhibitors in vertebrates and invertebrates. Insect serpins are key players in the defense mechanism of insects, especially the Toll pathway and PPO cascade. (**Figs. 7a & 7b**).

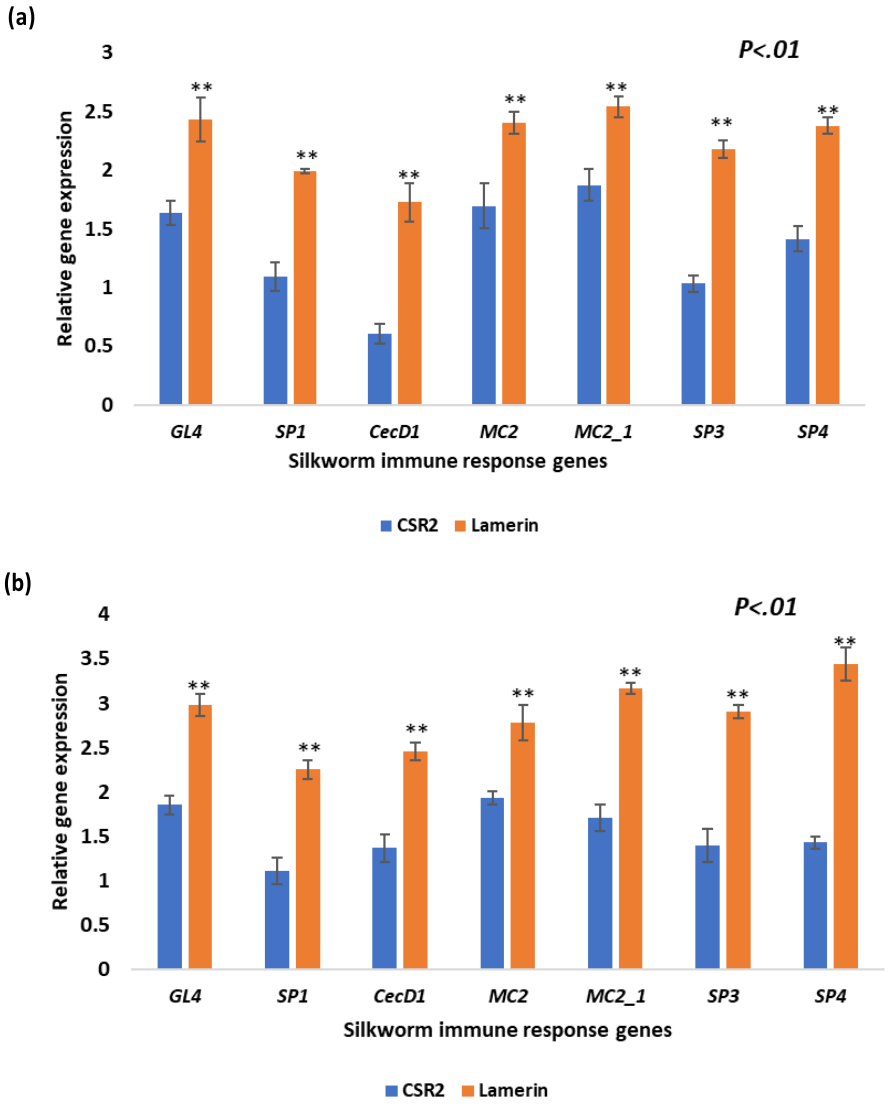

Figure 7: qPCR analysis of host gene responses against the *N. bombycis* infection at 24 hpi (a) and 48 hpi (b)

The work carried out in the report involves differential gene expression analysis of some of the host gene responses against *N. bombycis* infection that were identified through microarray and digital gene expression analysis. 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 CSR2 breed is an indicative of the tolerance / resistance exhibited by the Lamerin breed against the *N.bombycis* infection. The upregulation of immune genes in the Lamerin breed may be attributed to the genetic make-up of the breed and its adaptability. The future line of work involves transcriptome and miRNA analysis among both the breeds post infection with *Nosema bombycis* to study the changes in the expression levels of messenger RNAs (mRNAs) and non-coding RNAs, including microRNAs (miRNAs) following infection which will provide vital information on host defense strategies against invading pathogens.

4. PIT-08004MI: 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 is a prerequisite and vital. Homozygous / inbred lines are used for trait heritability studies and trait discovery applications such as mapping and gene functional analysis and for development of superior hybrids from the parents lacking undesirable alleles. Successful deployment of doubled haploids by production of haploids through androgenesis and / or gynogenesis adopting 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 that, the main limiting factors for initiation of microspore embryogenesis are low cellular reprogramming efficiency and high microspore / 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

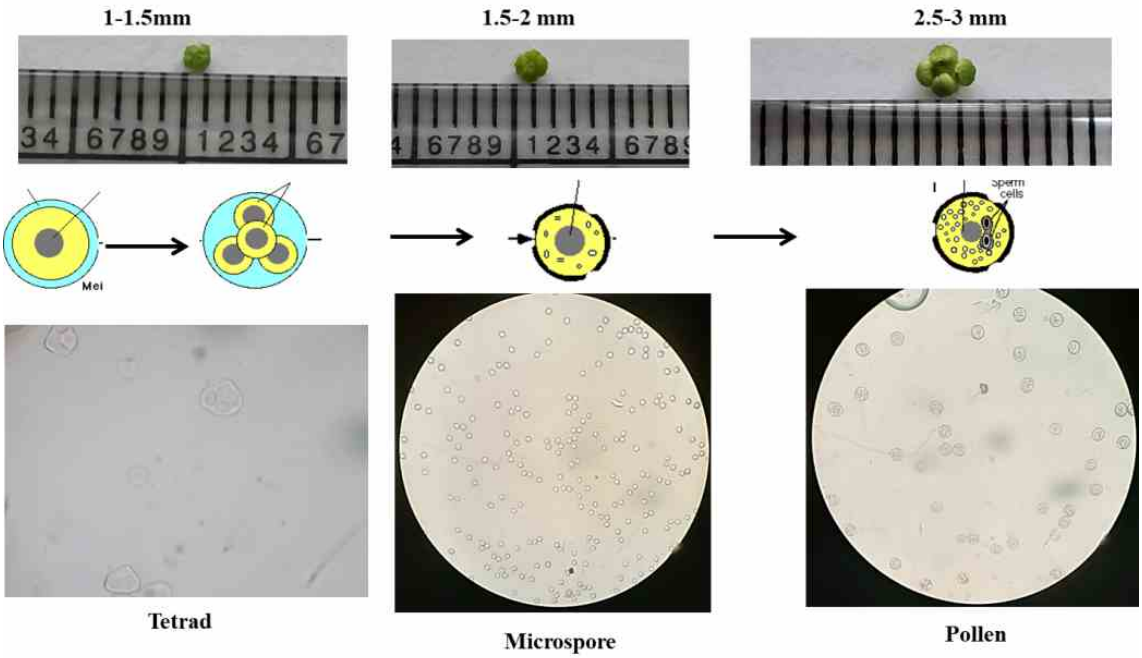

Figure 8: Flower bud size with the stage of microspores: The mulberry flower bud size of 1.0 to 1.5mm, 1.5 mm to 2mm and 2.5 mm to 3 mm correspond to tetrad, microspore and pollen stages respectively. Bud size of 1.5 mm to 2 mm suitable for isolating anthers and microspores for inducing embryogenesis.

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.

The stage of microspores / anther used as an explant for haploid embryogenesis is one of the determinant factors contributing to the success of developing haploid plants. To find out the stage of microspore with respect to development of flower buds, the morphology of the flower buds, catkin size and length were analyzed. We found that the bud size of 1.5 mm to 2 mm and morphology of the bud with anther lobes closed are ideal for haploid embryogenesis (**Fig. 8**).

The microspore isolation protocol was standardized to study effect of different pharmacological chemicals on microspore embryogenesis. Briefly, Catkins corresponding to desired bud size were collected early in the morning and surface sterilized. The buds were crushed and suspension filtered through 40 micron filter into the centrifuge tube. The filtered solution containing microspores was centrifuged at 4^{o}C for 5 min at 1500 rpm and the washing step repeated two more times. Finally, the pellet was re-suspended in embryo induction media at final concentration of 1 x 10^5 microspores / ml.

Anther culture from V1 variety mulberry was initiated with different basal media / sucrose concentrations and the effect of different hormones as well as light and dark incubation conditions on androgenesis was studied. Initial response of anther culture with increased size of anthers, revealed callus / embryo like growth in some of the treatments (**Fig. 9**).

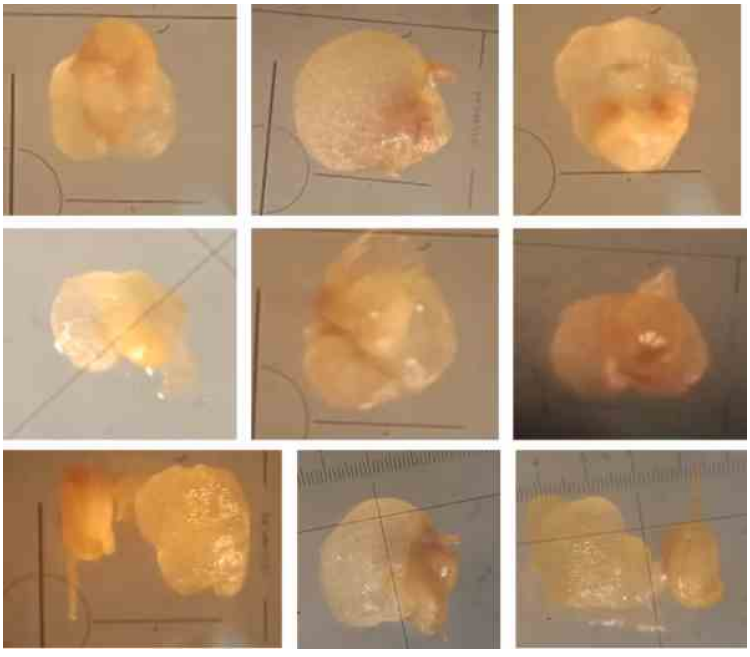

Figure 9: Callus / embryo induction from anther cultures of V1 variety

5. AIT-08005MI: 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* and 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.

The bivoltine / multivoltine parents which are used to produce commercial cross breeds such as Pure Mysore and Nistari and other productive breeds were screened for BmBDV resistant gene. The parents of the commercial breeds / hybrids in pipeline at CSR&TI, Mysore and CSR&TI, Berhampore were initially screened for the presence of resistant and susceptible *nsd-2* genes either in the homozygyous or heterozygous condition. Parental breeds with *nsd-2* gene in heterozygous condition were identified to be made into homozygous lines by sib mating within the breeds to prepare disease free layings (DFL). Further, genomic DNA will be isolated from the male and female moths of each DFL and PCR will be done using *nsd-2* resistant and susceptible primers. The DFLs with male and female parents homozygous for *nsd-2* resistant allele will be taken forward and brushed for continuing the subsequent generations and the developed lines will be tested using bioassay for BmBDV resistance. The shortlisted homozygous parental lines that are productive will be utilized to develop different combinations of hybrids and the shortlisted hybrids will be again evaluated by bioassay for BmBDV resistance. The selected hybrids after evaluation will be tested at RSRSs for assessing field performance and the hybrid combinations will be evaluated for BmBDV resistance.

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

a. Screening at larval stage

Larvae of 14 bivoltine as well as 05 Multivoltine breeds from CSR&TI, Mysore and 04 bivoltine as well as 05 Multivoltine breeds from CSR&TI, Berhampore **(Table 3)** were collected and checked for the presence or absence of the BmBDV resistant *nsd-2* gene using gene specific primers. Samples of 100 worms from each breed were taken and analysed for the presence of the resistant *nsd-2* gene by using the AA1 (Resistant) and AA3 (Susceptible) primers.

Out of total 18 bivoltine and 9 multivoltine breeds screened for the presence of AA1 *nsd-2* resistant marker, the bivoltine breeds S8, CSR50, 2C, 4D, 4S, 5HT, 8HT, DUN-17, D2, CSR-16, CSR-26N, B.con1, B.con 4 and all 9 multivoltine breeds showed amplification with only AA3 *nsd-2* susceptible marker (susceptible to BmBDV). None of the aforesaid breeds revealed amplifications for *nsd-2* resistant marker (BmBDV resistant). Therefore these breeds are considered to be homozygous susceptible as there is the absence of resistant allele. The races SK6, SK7, DUN-18, GEN-1 and CSR-16N showed amplifications with both AA1 *nsd-2* resistant (resistant to BmBDV) and AA3 *nsd-2* susceptible (susceptible to BmBDV) gene specific primers and therefore these races possess the resistant allele either in homozygous / heterozygous conditions. Hence, larvae of these breeds 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 to check the presence of marker in each DFL in either homozygous resistance / heterozygous condition (**Fig. 10**).

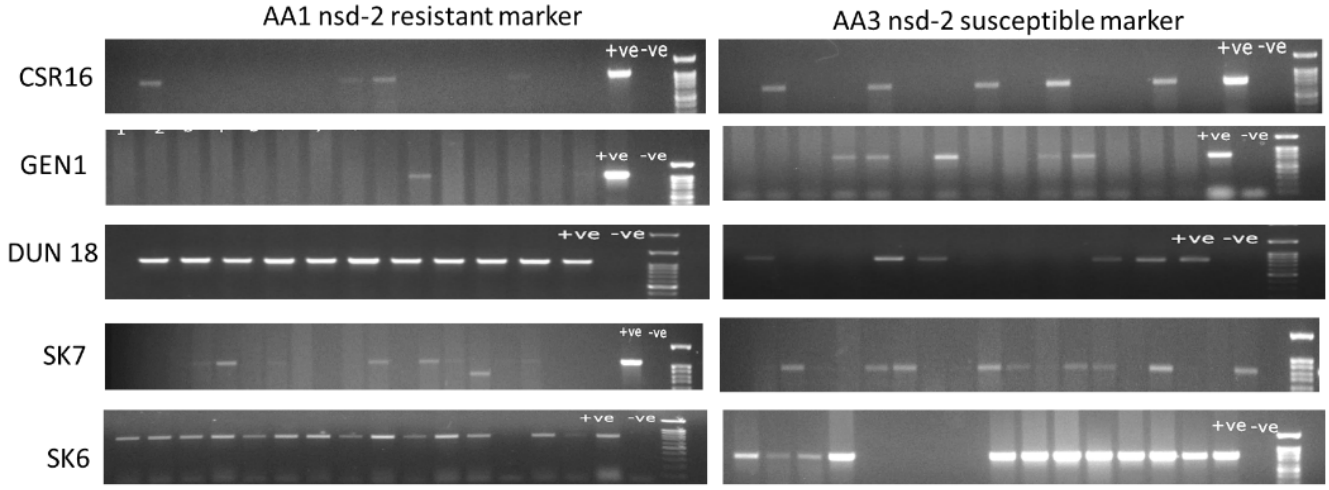

Figure 10: Screening of silkworm larva for the presence of BmBDV marker. The races that showed the presence of BmBDV resistant marker are only shown while the remaining races showed positive for BmBDV susceptible marker.

Table 3: Silkworm bivoltine and multivoltine races screened for the presence of BmBDV resistant marker

Sl. No.	Silkworm breed (Bivoltine)	Status of nsd-2 resistant allele
1.	S8 (O)	Absent
2	CSR50 (O)	Absent
3	CSR 16N(D)	Absent
4	CSR 26N (D)	Absent
5	Gen1 (O)	Present

Sl. No.	Silkworm breed (Bivoltine)	Status of nsd-2 resistant allele
6	2C (O)	Absent
7	4S (D)	Absent
8	4D (D)	Absent
9	5HT (D)	Absent
10	8HT (O)	Absent
11	DUN 17 (D)	Absent
12	DUN18 (D)	Present
13	CSR 16 (D)	Present
14	D2 (O)	Absent
15	SK6 (D)	Present
16	SK7 (D)	Present
17	B. con 1 (O)	Absent
18	B. con 4 (O)	Absent
Silkworm breeds (Multivoltine)		
1	M6DPC (y)	Absent
2	M12 W (y)	Absent
3	Nisatri 0017 (o)	Absent
4	Nistari 0018 (o)	Absent
5	Nistari 0019 (o)	Absent
6	PM (gy)	Absent
7	MV1 (gy)	Absent
8	ICB 29 (gy)	Absent
9	ICB 30(gy)	Absent

b. Screening at moth stage

The AA1 (BmBDV resistant) positive male and female moths of each DFL observed during larval screening were collected from the shortlisted breeds and tested for presence of BmBDV resistance marker using AA1(BmBDV resistant) and AA3 (BmBDV susceptible) primers to confirm presence of *nsd-2* alleles in either homozygous or heterozygous condition. Among the five breeds

tested, none possessed the *nsd-2* marker in homozygous condition. However, all the breeds showed presence of alleles in heterozygous condition with slight variation in the heterozygous percentage. The SK6 breed showed 25% heterozygosity ie., $+^{nsd-2}/nsd-2$ genotype, and 75% homozygosity for *nsd-2* resistant alleles, SK7 breed showed 86% hetrozygosity while remaining breeds revealed 14% homozygosity for *nsd-2* resistant alleles. Therefore, it can clearly observed (**Fig. 11**) that, SK6 breed DFLs of lines 1, 3, 4, 5, 7, 8 are completely homozygous, while, remaining DFLs showed heterozygosity. In SK7 breed, DFL of only line 3 showed the presence of *nsd-2* alleles in homozygous condition, while, DFLs of remaining lines showed heterozygosity. Screening of CSR16 and Dun18 breeds for selecting homozygous lines is in progress. The selected homozygous lines of SK6 and SK7 breeds were maintained under standard rearing conditions for performing bioassay with BmBDV.

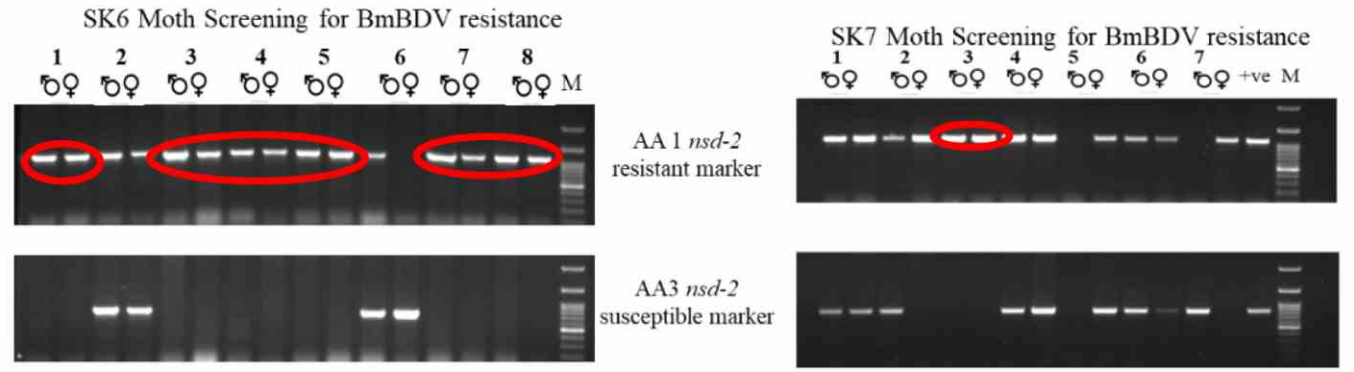

Figure 11: Screening of SK6 and SK7 silkworm moths for the presence of BmBDV marker. The races that showed the presence of BmBDV resistant marker in homozygous condition are shown in red

Transfer of resistance gene to CSR2 and CSR27 breeds and susceptible parents of commercial hybrids from West Bengal regions

The productive breeds CSR2 and CSR27 as well as parental breeds of FC2 are completely susceptible to BmBDV infection as they possess *nsd-2* alleles in homozygous condition. Therefore to develop BmBDV resistant lines of both the breeds, a donor parent was to be identified. Therefore, both CSR2 and CSR27 breeds were crossed with J2P BmBDV resistant donor parent and subsequent back crossing with respective parents is in progress which are in BC4F1 generation. The backcross population were screened in each generation for the presence of AAI *nsd-2* resistant allele. After completion of six generations, the respective CSR2 and CSR27 population will be sib-mated to identify homozygous *nsd-2* resistant lines.

6. AIT-08006EF: Development of lateral flow assay kit for detection of pebrine disease in silkworm

(Funded by BIRAC, New Delhi in collaboration with Bhat Biotech Ltd, Bangalore;
Duration: March 2021- January 2023)

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

[1]CMER&TI, Ladhoigarh; [2]CTR&TI, 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.

The project was initiated by identifying the potential gene candidates, its cloning and expression. Based on the expression profiles of *Nosema bombycis* infection, spore wall protein 1 (SWP1) and spore wall protein 3 (SWP3) were identified as potential gene candidates for development of the lateral flow assay kit (LFA) since, these were found to express early meront stage of infection. The spore wall proteins 1 and 3 correspond to SWP30 and 32 respectively, where SWP30 is an endosporal protein and SPW32 to be exosporal protein.

The primers for target gene sequences were designed based on the transcriptome data available for *Nosema assamensis* from an earlier laboratory study. The primers were used to amplify the SWP1 and 3 from *Nosema assamensis*. The genes were cloned and sequenced using pJET vector to confirm the integrity of the sequence. The confirmed sequences were restriction digested to obtain the coding gene. The coding gene was analysed for the ORF identification, including the insertion of appropriate stop codons. The gene was ligated into pET32a vector which was transformed into BL21 codon plus cells for protein expression. The protein expression was achieved through IPTG induction and the protein expression was confirmed through SDS PAGE. In addition, to SWP1 and 3, SWP5 which was previously established to be a surface protein was also expressed and purified. The SWP 1 and 3 expressed proteins were found to produce bands near 46kDa and 52kDa of the protein ladder for SWP1 and 3 respectively. The protein expression was performed in bulk to extract quantity required for antibody generation. The immunization in mice has been initiated and the bleeds from these mice will be used in further development of LFA kits.

OUTCOME OF CONCLUDED RESEARCH PROJECTS

1. ARP-3605: Validation of the DNA markers in silkworm breed developed by introgression of DNA markers associated with NPV resistance using Marker Assisted Selection Breeding and large-scale field trial of the breed

(Jointly with CSR&TIs of Mysore, Berhampore and Pampore and in technical collaboration with NSSO, Bangalore; **Duration:** Feb. 2017-Aug. 2020)

Co-Ordinator: Dr. V. Sivaprasad, Director, CSR&TI, Berhampore

A. R. Pradeep, K.M. Ponnuvel, Ms. Mariam Susan Joseph; S. Manthira Moorthy[#]; B. Mohan[&]; Gopal Chandra Das[1], N. Chandrakanth[1]; Sardar Singh[2], Mohamed Aslam[2] and Babu Lal**

[#]CSR&TI, Mysore; [&]SSBS, Coonoor; [1]CSR&TI, Berhampore; [2]CSR&TI Pampore-RSRS Jammu; **RSRS, Dehradun

Objectives

SBRL

1. Validation of DNA markers for NPV resistance and stress tolerance in selected lines being used for field trials.
2. Continuous maintenance of MAS-N Lines; Co-ordination and statistical analyses of observations from lines reared at different stations.

CSRTI Mysore, Berhampore and Pampore

1. To evaluate the evolved bivoltine lines in various agro climatic conditions and select lines for their suitability in that particular environment
2. Preparation of Dfls of Pure Mysore or Nistari x MASN and MASN x CSR4 through NSSO, Bangalore and distributed to Sericulture Farmers of area under CSRTIs.

Improvement of sericulture in the country depends on synthesizing new breeds with higher yield and better disease tolerance by crossing mulltivoltine x bivoltine and bivoltine x bivoltine breeds. Notably, multivoltine breeds are pathogen tolerant in comparison to bivoltine races, whereas, the bivoltine breeds are with higher cocoon yield traits including better post cocoon traits than multivoltine breeds. Developing a breed with better pathogen tolerance coupled with high yield traits is the ultimate aim of silkworm breeding. Anticipating best heterosis after cross, the multivoltine breed Sarupat was identified as most nucleopolyhedra virus (NPV) tolerant breed and the bivoltine breed CSR2 as most NPV susceptible breed through screening silkworm germplasm accessions available at CSR&TI, Mysore, Karnataka State Sericultural Research and Development Institute (KSSRDI), Bangalore and Andhra Pradesh State Sericultural Research and Development Institute (APSSRDI), Hindupur.

In order to find the genetic polymorphism, more than 800 primers of dominant and co-dominant markers were tested. Three genic markers developed from protein kinase G-1B (Nag65), glucosyl transferase (Nag 84) and deoxy kinase (Nag 88) by CDFD (by a group of late Dr. J. Nagaraju) showed consistent polymorphism between Sarupat and CSR2 breeds. On inheritance analysis of F2 of Sarupat x CSR2 cross, few (1 – 2) alleles from each parent showed inheritance and statistical association with tolerance level. The NPV tolerant gene markers, Nag 65, Nag 84 and Nag 88 were associated with NPV tolerance, mapped in chromosomes 05, 07 and 03, respectively and were used for marker assisted selection (MAS) and breeding programs through five back cross generations (BC5) [(BC1 is (Sarupat x CSR2) x CSR2)]. During each generation, artificial selection was employed to select lines for higher yield and better cocoon traits. These lines were further tested for NPV tolerance in alternate generations through experimental NPV infection and bioassay. Out of the selected batches, lines that showed 50 – 60 % survival after experimental infection (tolerant) and with higher yield traits were selected. After five BC generations, selection was continued in sib-mated (BC5 x BC5) generations (BC5F1). Three bivoltine lines *viz.*, MASN-4, MASN-6 and MASN-7 (MASN- marker assisted selection lines for NPV tolerance) with better NPV tolerance and high cocoon yield traits were segregated after five more generations. The lines have been maintained upto 30 generations (BC5F30) along with the selection process and used as parents for hybrid preparation. The F1 generation of Sarupat x CSR2 had silk ratio (SR%) of 20.88 ± 1.2% which was improved to 22.78 ± 1.08 % after five back crosses (BC5). After 30 generations of sib-mating (BC5F30) the lines are stabilized and SR% reached 20.86 ± 3.83% (range 20 – 24%).

Synthesis of hybrids for field tests

In order to synthesize F1 progeny hybrids, female moths of MASN lines were crossed with potential high yielding bivoltine male moths of CSR4 (bivoltine hybrid: MASN4 x CSR4). In addition, cross breeds of the MASN lines were developed by crossing female moths of the multivoltine breeds Pure Mysore (PM) and Nistari with male moths of MASN4 (Nistari x MASN4 and PM x MASN4). These bivoltine hybrids and MV x BV cross breeds were distributed among the farmers for field testing in South India, East India and North-West India.

Marker evaluation

The MASN lines were evaluated for presence of genic markers associated with NPV tolerance developed from deoxy kinase (Nag 88), protein kinase G-1B (Nag65) and glucosyl transferase (Nag 84). In nearly 90% individuals of pure MASN lines, markers were present and only those individuals with the markers were used as parental (P1) generation to develop hybrids / cross breeds. Among the lines, MASN4 showed better yield traits than MASN6 and MASN7 lines. In addition to SBRL, MASN lines have also been maintained at Silkworm Breeding station, Coonoor and these batches are tested for marker presence before using them as parents to develop F1 hybrids for field supply.

Expression analysis of Nag 65, Nag 84 and Nag 88 genes showed enhanced expression in MASN lines in comparison to parental races (**Fig. 12**).

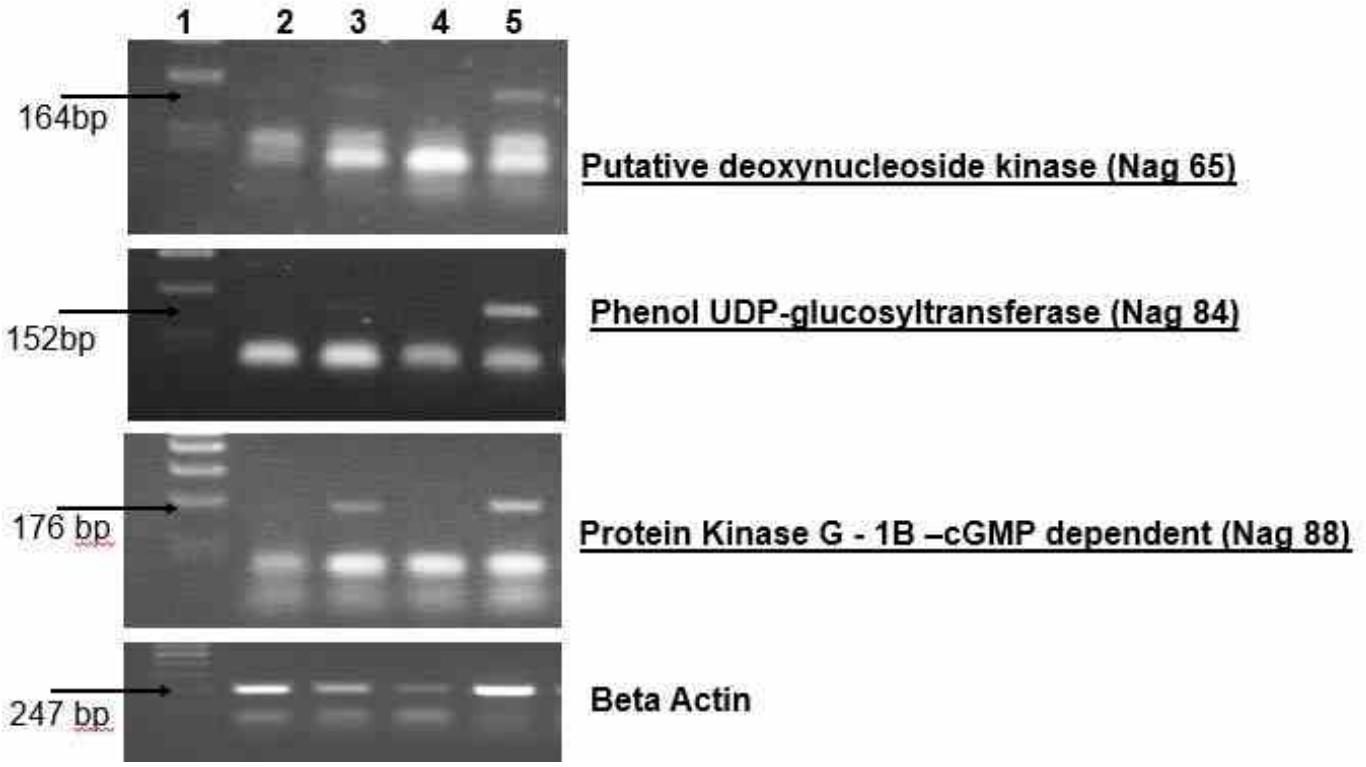

Figure 12: RT-PCR showing differential expression of NPV tolerance associated genes among Sarupat (Lane 2), CSR2 (lane 3), CSR4 (lane 4) breeds and MASN4 (lane 5) line: Lane 1 is DNA ladder (marker). Sarupat and CSR2 breeds are parental races of MASN4 line. The gene expression was analysed in the midguts of day 5 of non-infected fifth instar larvae showing enhanced expression in MASN4 line.

Presence of the genic markers was tested in the F1 hybrids developed for different stations at Jammu, Dehradun, West Bengal and South India. Nearly 85% individuals of different batches showed uniform presence of the markers (**Table 4**). In order to test segregation of the markers, F2 generation was developed from the crosses. Segregation of the alleles for NPV tolerance associated genes was observed for Nag 84 and Nag 88, whereas, single marker was observed with Nag 65 showing independent segregation of the markers (**Fig. 13**). In order to test whether nucleotide sequences of the alleles are similar to the gene sequence, the allele bands were cloned and sequenced. The sequence of Nag 65 allele from crosses of Nistari and PM breeds with MASN4 line, MASN4 x CSR4 and CSR2 x CSR4 hybrids showed similarity with *B. mori* and *B. mandarina* deoxynucleoside kinase (expect value 2e-75 with 98 to 99 % identity; **Fig. 14**).

Table 4: Marker presence in different hybrids developed from MASN4

Marker	Hybrids / cross	Hybrid	Total Individuals	Individuals with markers	Individuals without markers	% without markers
NAG65	MASN4 X CSR4	F1	123	110	13	10.57
	NISTARI X MASN4	F1	31	26	5	16.13
	PM X MASN4	F1	94	87	7	7.45
NAG84	MASN4 X CSR4	F1	113	101	12	10.62
	NISTARI X MASN4	F1	56	48	8	14.29
	PM X MASN4	F1	31	24	7	22.58
Nag88	MASN4 X CSR4	F1	71	54	17	23.94
	NISTARI X MASN4	F1	41	31	10	24.39
	PM X MASN4	F1	70	58	12	17.14
					Average	**16.35%**

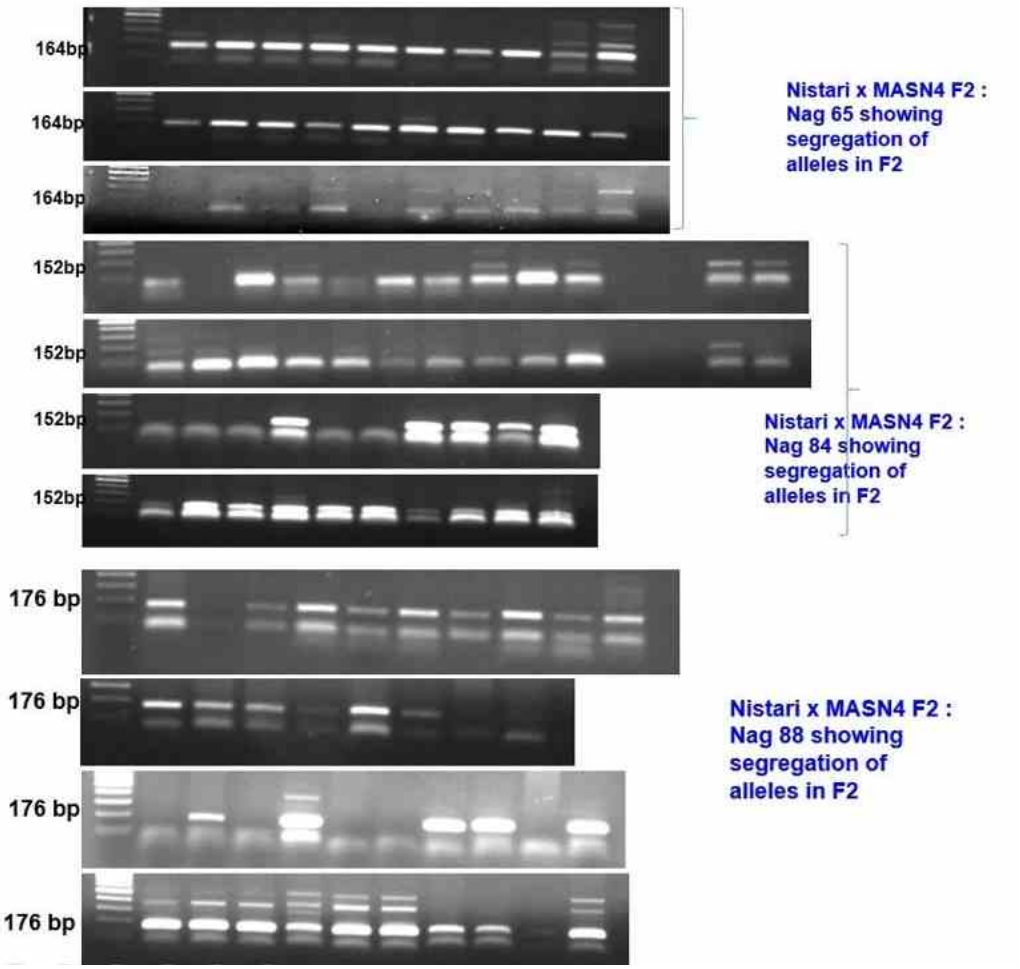

Figure 13: Segregation of alleles in F2 generation developed from Nistari x MASN4

Description	Max Score	Total Score	Query cover	E Value	Per. Ident	Accession
PREDICTED: Bombyx mandarina deoxynucleoside kinase-like...	298	298	92%	3e-78	99.39	XM_028175711.1
PREDICTED: Bombyx mandarina deoxynucleoside kinase-like...	298	298	92%	3e-78	99.39	XM_028175710.1
PREDICTED: Bombyx mandarina deoxynucleoside kinase-like...	298	298	92%	3e-78	99.39	XM_028175709.1
PREDICTED: Bombyx mandarina deoxynucleoside kinase-like...	298	298	92%	3e-78	99.39	XM_028175708.1
PREDICTED: Bombyx mandarina deoxynucleoside kinase-like...	298	298	92%	3e-78	99.39	XM_028175707.1
PREDICTED: Bombyx mori deoxynucleoside kinase-like	287	287	92%	6e-75	98.17	XM_012695612.2
PREDICTED: Bombyx mori deoxynucleoside kinase-like	287	287	92%	6e-75	98.17	XM_012695611.2
Bombyx mori mRNA, clone: fcaL07I23_K01581	287	287	92%	6e-75	98.17	AK384095.1
Bombyx mori deoxynucleoside kinase-like (LOC692569), mRNA	287	287	92%	6e-75	98.17	NM_001043554.1

Figure 14: BLAST analysis of Nag 65 allele sequence showed nearly 98% identity with deoxynucleoside kinase sequence from *Bombyx mandarina* and *B. mori*.

However, 6 % nucleotide variations were observed between Nag 65 alleles of CSR2 x CSR4 (query 65C1) and MASN4 x CSR4 hybrids (Sbjct 65M1) (**Fig. 15**).

```
Query  1    CCCGCCG-ATGTCGGGAATGGCTCGAAGGTTGTTCAGCAAGATTTCCTTGACGATGTTGC  59
            ||||| | ||  |||||| |||||||  ||| || |||||||||| ||||||||||||||
Sbjct  1    CCCGCGGGATTCCGGGAA-GGCTCGA--GTTTTT-AGCAAGATT-CCTTGACGATGTTG-  54

Query  60   GACATGCACCGGAGACCTGCTCCAATTCCAGTAAAGCTAATGGAGCGATCATTATTCAGT  119
            |||||||||||||||||||| ||| ||||||||||||||||||||||||||||||||||
Sbjct  55   GACATGCACCGGAGACCTGCTTCAACTCCAGTAAAGCTAATGGAGCGATCATTATTCAGT  114

Query  120  GCGAGATACTGCTTCGTTGAACACATGATGAGAAATAATACACTCCATCCAGCACAGTTT  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  GCGAGATACTGCTTCGTTGAACACATGATGAGAAATAATACACTCCATCCAGCACAGTTT  174

Query  180  GCAGTACTTGATGAGTGGTTCCGATTCA  207
            ||||||||||||||||||||||||||||
Sbjct  175  GCAGTACTTGATGAGTGGTTCCGATTCA  202
```

Figure 15: Alignment of sequences of Nag 65 alleles from CSR2 x CSR4 (query 65C1) hybrid and MASN4 x CSR4 hybrids (Sbjct 65M1) showing 6% variability

On alignment of nucleotide sequence of Nag 84 alleles from MASN4 x CSR4 (84M1; subject) and CSR2 x CSR4 (84C1, query) hybrids, the sequences showed only 48% alignment indicating 52% dissimilarity.

Maintenance of MASN breeds

MASN lines are maintained through induction of diapause by four and six month long egg preservation schedule. Mature larval weight was in the range of 3.4 to 3.6g, whereas, silk (shell) ratio was 18 to 23% (**Table 5**). In general, the larval weight and cocoon traits of MASN lines did not show significant ($P < 0.51$; NS) difference from CSR lines indicating that MASN lines attained yield traits of CSR lines.

Performance of MASN lines at different centres in South India was uniform and did not show significant (P < 0.98 ANOVA) variation showing adaptability under different climatic zones. However yield of MASN4 line was significantly higher (CD at 5% was 0.003) than other lines. The reeling parameters were also tested for the batches raised at two stations, Bangalore and Jammu. Significant variations were not observed (P < 0.9) between the two batches.

Table 5: Cocoon traits of MASN lines at SBRL, Bangalore

TRAITS	MASN-4	MASN-6	MASN-7	CSR2	CSR4
Larval weight	3.456 ± 0.237	3.569 ± 0.217	3.398 ± 0.291	3.438 ± 0.190	3.512 ± 0.213
Cocoon weight	1.454 ± 0.214	1.573 ± 0.226	1.459 ± 0.180	1.495 ± 0.157	1.693 ± 0.334
Shell weight	0.267 ± 0.032	0.347 ± 0.196	0.328 ± 0.140	0.316 ± 0.028	0.318 ± 0.0487
Shell Ratio (%)	18.611 ± 2.549	22.947 ± 2.105	22.932 ± 2.973	21.277 ± 2.331	18.913 ± 0.864

Six rearing trials of MASN lines (4, 6 and 7) were performed at CSR&TI Berhampore in different seasons [favourable seasons (October to March); unfavourable seasons (April to September) in West Bengal]. Fecundity, hatching and survival % showed uniformly higher estimate for MASN4 line. The performances of the MASN lines during favourable seasons showed better cocoon parameters and survival % (68 – 78% ERR) than its control CSR2 breed (40-50% ERR). During unfavourable season, the control CSR2 breed did not survive in this region, whereas, MASN lines showed survival of 40-50 %. CSR2 breed showed low Effective Rate of Rearing (ERR) as 1313 / 10000 larvae indicating 13% survival in February- March, whereas, no survival was reported in summer.

The evolved MASN-4, MASN-6 and MASN-7 lines were supplied by SBRL to both RSRSs in Dehradun and Jammu during March-April 2017. Spring rearing was successful at Jammu with survival was upto 90%, whereas, Autumn rearing showed only 20-30% survival; however survival improved during autumn crop 2018. Average yield in Autumn 2017 was 15 to 20 Kg/ 100 DFLs, whereas, in Autumn 2018, it increased to 57 – 59 Kg/ 100DFLs showing faster adaptability. Similarly, fecundity was higher (450-500) in both the seasons and shell ratio was 21 - 22% in all MASN lines during spring. The pupation rate was 91 - 92% in Spring, 2017, whereas, in 2018, it was 95% showing better survival.

The reeling parameters of MASN lines maintained at Bangalore and that reared at Jammu did not show significant (P < 0.98; NS) differences showing uniformity in attaining post cocoon traits under different conditions (**Table 6**).

Table 6: Reeling parameters of P1 MASN lines at two stations

Center	P1	Average filament length (m)	Reelability %	Raw silk %	Waste %
Jammu	MASN-4	572	72.46	26.51	26.47
	MASN-6	947	73.69	27.53	22.86
	MASN-7	711	78.23	33.52	20.93
Bangalore	MASN-4	697	91.7	34.2	22.7
	MASN-6	702	92.5	34.2	22.7
	MASN-7	810	90.8	34.6	16.7

Comprehensive data from different regions showed stabilized fecundity in South India and West Bengal, whereas, it was significantly (P < 0.009) less in Dehradun and Jammu. Among the fitness traits, egg hatching showed uniformity in all the southern and northern regions indicating the fitness potential of the lines under different seasons in different regions (**Fig. 16**).

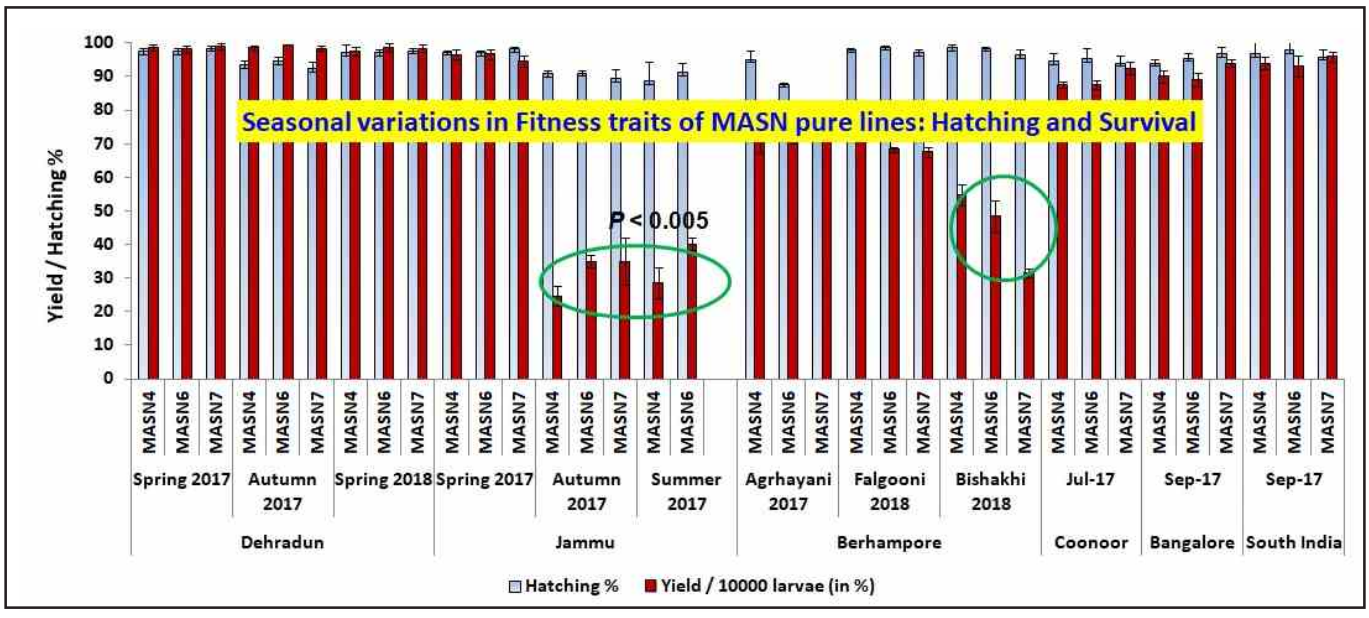

Figure 16: Yield / egg hatching % (Mean ± SD) variations among MASN lines in different regions in different seasons. Significant variations are encircled

In Spring season of Dehradun and Jammu as well as in Agrahayani (October-November) and Falgooni (February – March) crops of West Bengal, ERR i.e., yield per 10000 larvae was higher (70 - 90%) showing adaptability of the MASN lines in favourable seasons in different regions. However, the ERR significantly ($P < 0.005$) reduced during Autumn and Summer seasons in regions of Jammu and during early Summer season in West Bengal (Bishakhi) in sericulture belt indicating the environmental interaction on larval growth.

The adaptive traits, cocoon weight and silk (shell) weight showed uniformity in South India. It decreased during spring and autumn seasons of Dehradun and Jammu, whereas, the silk content (silk ratio %) did not show significant ($P < 0.08$) variation among the seasons as well as among the regions revealing the stability in these adaptive traits in MASN lines.

Synthesis of hybrids / cross breeds and distribution for field evaluation in geographical regions under different climatic conditions of South and North India

The MASN lines were developed from the cross of Sarupat female and CSR2 male in which Sarupat is a disease tolerant low yielding multivoltine breed and CSR2 a disease susceptible high yielding bivoltine breed. The F1 generation of Sarupat x CSR2 had silk ratio (SR%) of 20.88 ± 1.2% which improved to 22.78 ± 1.08% after five back crosses (BC5). Presently, after 30 generations (BC5F30), the lines have stabilized and SR% reached 20.86 ± 3.83% (range 20 – 24%). However, SR% of the control cross CSR2 x CSR4 had a higher peak of 23%. Achieving this level is difficult as both CSR2 and CSR4 parents are highly susceptible to pathogens revealing that MASN lines are with predictably better tolerance level as well as with stabilized high yield parameters. In order to prepare F1 hybrids, crosses of Nistari x MASN4, PM x MASN4 and the bivoltine hybrid MASN4 x CSR4 were made that showed 95 – 99 % survival under laboratory rearing conditions. These larvae showed better yield, cocoon traits and survival and are used for field evaluation.

Among the hybrids / cross breeds, MASN x CSR4 showed 55 - 68 % mortality after experimental infection with 6000 polyhedra per larva, whereas, the control cross CSR2 x CSR4 showed significantly higher (90%) mortality (**Fig. 17**). The multivoltine cross PM x MASN4 showed 62% mortality which was marginally ($P < 0.0778$) higher than PM x CSR2. Nistari x MASN4 showed ~55% mortality equivalent to control cross Nistari x CSR2. As a whole, MASN based breeds showed comparatively higher survival post NPV infection in comparison to the control CSR crosses / hybrids.

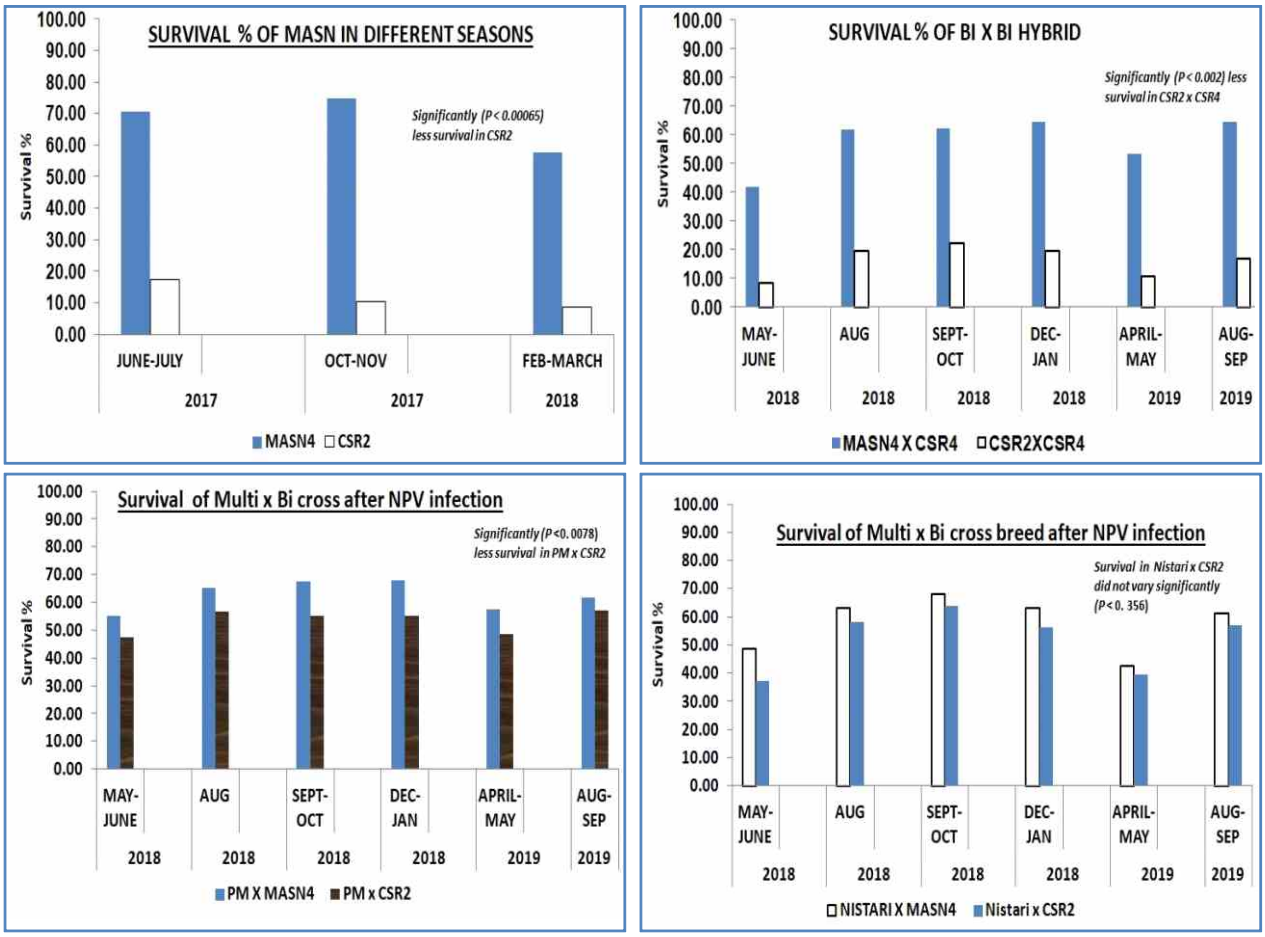

Figure 17: Survival of MASN lines and its hybrids after infection with NPV (6000 polyhedra / larva) under different seasons of Bangalore (*n* = 300 each)

Crosses were made between PM and MASN4 as well as Nistari and MASN4. The cocoons of MASN hybrids / crosses (**Fig. 18**) were with strong built up of significantly (P < 0.05) higher cocoon, shell and pupa weight than that of the ruling breeds. Similar results were obtained at different regions (**Table 7**). Shell ratio (%) was 19- 20% in West Bengal and 20-22% in Jammu and Himachal Pradesh. Cocoon traits of the MASN hybrids / crosses showed significant increase when compared to ruling varieties in different regions (**Table 7**).

In order to test differences in reeling parameters among the crosses from the three lines, reeling test was conducted at Central Silk Technology Research Institute (CSTRI, Bangalore). Significant differences were not observed among the bivoltine hybrids developed from MASN4, MASN6 and MASN7 lines.

The MASN cross breeds and bivoltine hybrids were distributed in different regions of south and north India. In Jammu, the yield was 46.50 Kg better than the ruling hybrid FC1 x FC2 (39.35 kg), whereas, in Himachal Pradesh yield increased to 51.36 kg.

Figure 18: Cocoons of parental races, MASN4, CSR4, Pure Mysore and Nistari and Nistari x MASN4 and PM x MASN4 crosses and MASN4 x CSR4 hybrid developed from the parents

Table 7: Cocoon traits of different crosses and hybrids developed from MASN4 (Sep-Oct 2018): Performance at different regions

Region	Hybrid / Cross	cwt	swt	pwt	SR %
Mysore	**PM x MASN4**	**1.817 ± 0.345***	**0.333 ± 0.047**	**1.4977 ± 0.304**	18.616 ± 2.623
Mysore (control)	PM x CSR2 (control hybrid)	1.531 ± 0.231	0.297 ± 0.0327	1.233 ± 0.278	19.41 ± 2.879
Berhampore	**Nistari x MASN4**	**1.858 ± 0.401**	**0.358 ± 0.084**	**1.353 ± 0.364**	**19.294 ± 5.395**
Berhampore (control)	Nistari x (SK6 x SK7) (Ruling cross breed)	1.506 ± 0.391	0.246 ± 0.074	1.260 ± 0.401	16.33 ± 3.867
Berhampore	**MASN4 x CSR4**	**1.889 ± 0.311**	**0.382 ± 0.0427**	**1.507 ± 0.288**	**20.22 ± 3.43**
Berhampore (control)	SK6 X SK7 (Ruling hybrid)	1.487 ± 0.281	0.274 ± 0.035	1.213 ± 0.0007	18.43 ± 0.502
Jammu	**MASN4 x CSR4**	1.650 ± 0.075	0.330 ± 0.0135	1.320 ±0.065	19.91 ± 0.552
Himachal Pradesh	**MASN4 x CSR4**	**1.72 ± 0.025**	**0.391 ± 0.007**	**1.329 ± 0.017**	22.74 ± 0.084
Jammu / HP (control)	FC1 x FC2 (Ruling hybrid)	1.686± 0.053	0.384 ± 0.003	1.3006 ± 0.049	22.81± 0.485
Mysore	**MASN4 x CSR4**	1.681± 0.066	0.356 ± 0.022	1.325 ±0.044	21.13 ± 0.52
Mysore (control)	FC1 x FC2 (Ruling hybrid)	1.756 ± 0.0795	0.39 ± 0.022	1.366 ± 0.058	22.2 ± 0.29

CWT: cocoon weight; SWT: shell weight; PWT: Pupa weight; SR: Silk (shell) ratio %

***BOLDED traits estimates are significantly (*P* < 0.05; T- test) higher in MASN hybrids than ruling hybrids**

Controlled rearing under RSRS Jammu revealed increase in yield upto 75kg/100DFLs showing the potential and suitability of the MASN derived bivoltine hybrids for Jammu and Himachal Pradesh regions compared to the ruling breeds (**Table 8**). Both Jammu (300-400 msl) and Himachal Pradesh (450 – 6820 msl) are located at higher altitudes and the MASN hybrids may be suitable for survival in high altitude conditions. In West Bengal, MASN4 x CSR4 hybrid showed 57.13kg yield/ 100 DFLs which was higher than the ruling hybrid (SK6 x SK7) (52.90 kg) showing the suitability of MASN hybrid in West Bengal (**Table 8**).

Table 8: Yield output of MASN hybrids (Bi xBi and Multi x Bi) vs ruling hybrids in different regions

State	Bi x Bi Hybrid	No. of DFLs	Yield per 100 DFL	Multi x Bi Cross	No. of DFLs	Yield per 100 DFL
Andhra Pradesh	MASN4 x CSR4	31600	64.00	PM x MASN4	4500	62.09* (6.26%)
(control)	Ruling hybrid (FC1 x FC2)	12000	70.00	Ruling hybrid PM x CSR2	4350	58.20
Tamil Nadu	MASN4 x CSR4	12650	62.00	PM x MASN4	—	—
(control)	Ruling hybrid (FC1 x FC2)	10000	74.00	PM x CSR2	—	—
Karnataka	MASN4 x CSR4	43500	60.50	PM x MASN4	59000	54.00
(control)	Ruling hybrid (FC1 x FC2)	16500	66.00	Ruling hybrid PM x CSR2	3500	66.00
West Bengal	MASN4 x CSR4	24200	**57.13*(7.4%)**	Nistari x MASN4	25650	**51.88 (9.02%)**
(control)	Ruling hybrid SK6 x SK7	4000	52.90	Ruling hybrid Nistari x (SK6xSK7)	4300	47.20
Jammu Division	MASN4 x CSR4	10000	**46.50*(15.38%)**	—	—	—
Jammu RSRS	MASN4 x CSR4	100	**75.40*(47.81%)**	—	—	—
(control)	Ruling hybrid (FC1 x FC2)	12350	39.35	—	—	—
Himachal Pradesh	MASN4 x CSR4	11400	**51.36NS**	—	—	—
(control)	Ruling hybrid (FC1 x FC2)	70700	**51.68**	—	—	—

*** Significantly higher at *P* < 0.05; NS- non- significant but equal to ruling hybrid; % increase above the ruling hybrids is given in parentheses**

On analysis of reeling parameters, MASN4 x CSR4 hybrids did not show significant ($P < 0.9$; ANOVA) difference in the reeling parameters among different regions (**Table 9**).

Table 9: Reeling parameters of MASN hybrids from different regions

Region	BV Hybrid / MV Cross	Cocoon weight (g)	Shell ratio (%)	Unbroken Filament length (m)	Denier	Renditta	Silk recovery %
South India (Bangalore)	MASN4 x CSR4	1.839 ± 0.066	23.10	797	3.54	5.6	77.8
	PM x MASN4	1.817 ± 0.345	18.61	671	3.09	6.6	80.6
West Bengal	MASN4 x CSR4	1.889 ± 0.311	20.22	798	2.54	8.09	76
	NISTARI x MASN4	1.858 ± 0.401	19.29	726	2.53	8.31	72.8
Jammu	MASN4 x CSR4	1.650 ± 0.075	19.89	878	2.735	3.44	—
Himachal Pradesh	MASN4 x CSR4	1.72 ± 0.025	22.74	947	2.46	3.15	—

Outcome and utility of the project

Hybrids of Marker Assisted Selection lines (MASN) showed better or *at par* performance in North and North-West India compared to ruling hybrids indicating their potential for survival and maintaining yield traits under stress conditions. Molecular marker based bivoltine hybrids and Nistari x MASN cross breeds were developed which are suitable for rearing in north (West Bengal) and North-West (Jammu and Himachal Pradesh) India. The hybrids have to be authorized and recommended for distribution to farmers.

2. ARP-3606: Development of diagnostic tool for early detection of baculovirus causing tiger band disease in *Antheraea proylei*

(Funded by DBT, New Delhi, in collaboration with RSRS, Imphal; **Duration:** Feb. 2017-Aug. 2020)

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*RSRS, Imphal, Manipur

Objectives

1. To characterize the baculovirus pathogen causing tiger band disease in Oak tasar silkworm, *Antheraea proylei*
2. To study the pathogenesis, source and mode of infection of viral pathogen
3. To develop DNA based diagnostic tools for early detection of baculovirus causing tiger band disease

A. mylitta, the oak tasar silkworm of India, is commonly affected by virosis characterised by shrinking body of the infected larvae, generally occurring in the 5th instar close to the cocooning stage of the life cycle (**Fig. 19**). The infected silkworms show signs of poor eating and become sluggish and they also lose the ability to hold onto the twigs dropping down to the ground. The disease occurs throughout the year especially in rainy (Jun - Sep) and autumn (Sep - Oct) seasons. The disease

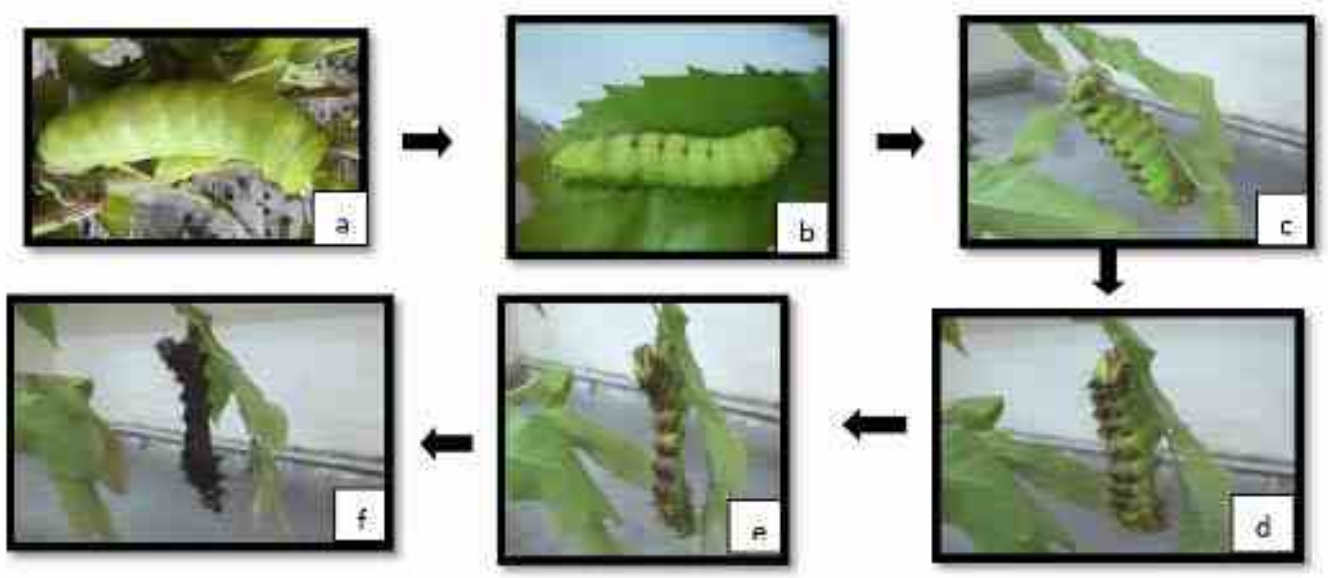

Figure 19: Infected *A. proylei* silkworm. (a) Healthy larva; (b) Initially black spots appear on dorsal body segments; (c-d) The black spots spread all over the body segments; (e-f) The whole body become black and larva died.

was previously suspected to spread mainly through droppings of other infected silkworms, disintegrated silkworms and pathogen contaminated rearing sites and appliances. High temperature, poor leaf quality of feed and high humidity is found to aggravate this disease. As the infection

progresses, the diseased silkworms show retarded growth, lethargy and refusal to feed. Eventually the larvae vomit the gastric contents which is followed by death. The causal virus for the disease occurrence, its etiology and pathogenesis were unknown earlier. Now we know that the disease is caused by group 1 alpha baculovirus called *A. proylei* nucleopolyhedro virus (*Anpr*NPV) which has been characterised at the genome level with complete genome sequencing and genes mapped / annotated (*Accession No. LC375539*). The genomic sequence shares 95% sequence identity with *A. pernyi* nucleopolyhedro virus (*Anpe*NPV).

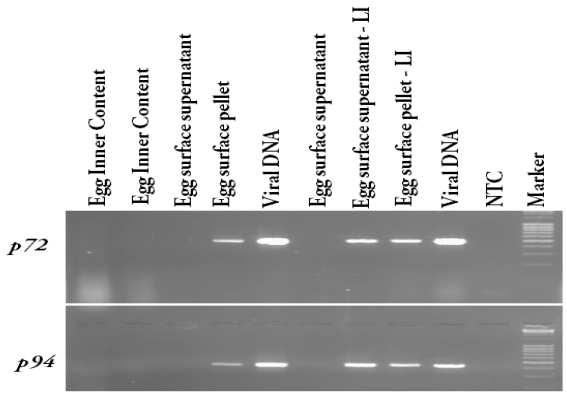

Figure 20: PCR analysis of the DNA samples before and after treatment with surface disinfection

Survey and pathogenesis

Based on the objectives of the study, a survey was conducted and diseased samples were collected for further activities. The data revealed that, disease occurrence was higher in spring and autumn seasons across various locations of Manipur and Nagaland. The source of virus was observed to the egg surface which were contaminated with the virus. This was confirmed before and after treatment of the egg samples with surface disinfection followed by PCR analysis of DNA samples using virus specific primers (**Fig. 20**). The absence of the virus in the inner content of the surface treated eggs was the proof to the hypothesis that the virus is transmitted through the surface contaminated eggs.

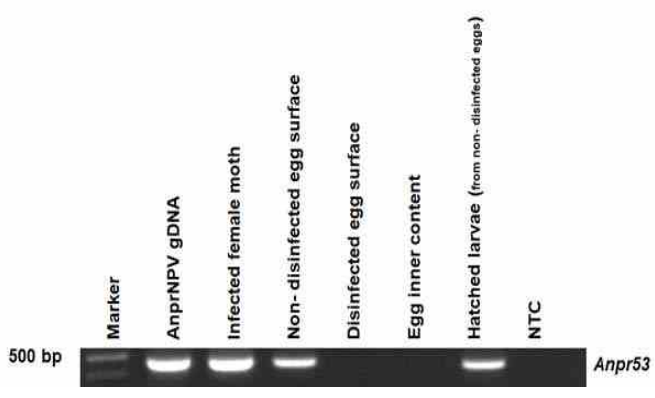

Figure 21: PCR analysis of the DNA samples before and after treatment with surface disinfection

The virus tissue tropism was also mapped based on qPCR analysis of various tissue samples (mid gut, Malpighian tubules, fat body, trachea, ovary) as well as development stages (larvae, pupae, moth egg, egg shell) of the infected silkworms. The virus was present in all the tissues and stages indicating its capability to establish a systemic infection in silkworms (**Fig. 21**). However, the most favourable tissue for multiplication was found to be fat body since, a higher copy number ($1x10^9$) of the virus was detected in these tissues.

The study also explored cross-infectivity of the *Anpr*NPV to other lepidopteran pests such as *Phalera raya*, *Hablaea peura*, *Peiris candidia* and *P. brassicae* which were unaffected by virus inoculation (**Fig. 22**). However, cross-infection to the other silkworms *A. frithi*, *A. pernyi* and *Samia*

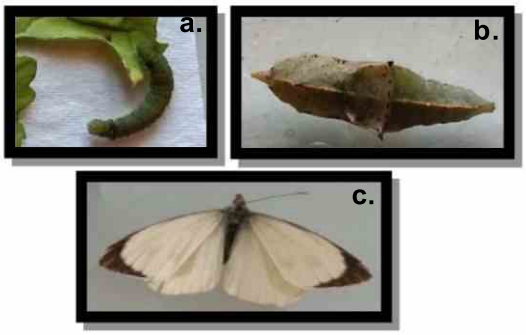

Figure 22: Cross infectivity analysis.
a. *P. canidia* larvae fed with AnprNPV;
b. Normal Pupa,
c. Normal adult

Figure 23: Cross infectivity analysis. a. Normal larvae of *Samia ricini,* b. Infected larvae

ricini, resulted in their death indicating that these saturniid silkworms are highly susceptible to infection (**Fig. 23**).

Disease control egg surface disinfection

Egg surface disinfection technique with 0.2% Sodium hypochlorite solution has been developed which, has shown to remove 100% virus from surface contaminated eggs. The method was demonstarted and the local DOS trained on surface disinfection of the eggs in Manipur and Nagaland.

Recently, with the help of NIMHANS, Bangalore TEM (Trasmission Electron Microscopy) images were obtained for *Anpr*NPV derived from infected *A. proylei* silkworms as a support of further characterization of the virus at the molecular level. The image shows the presence of occlusion bodies within an outer envelop, a typical organisation observed for viruses under *baculoviridae* (**Fig. 24**).

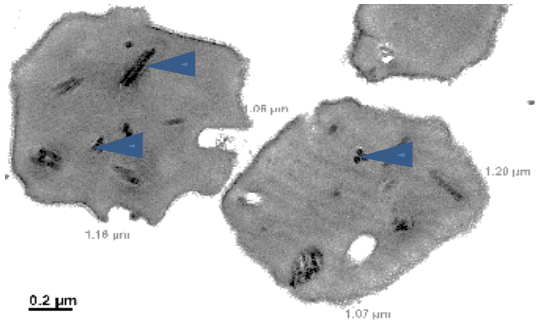

Figure 24: TEM image of occlusion bodies of the *Anpr*NPV

MEETING / WORKSHOP / CONFERENCE / SYMPOSIUM / SEMINAR

RAC Meeting conducted

The 26th meeting of the Research Advisory Committee was held on 7th January 2020 wherein review of the outcome of concluded projects and evaluation of new project proposals and progress of ongoing projects was conducted under the Chairmanship of Dr. N.K. Krishna Kumar, Former DDG (Horticulture), ICAR, New Delhi.

Brain storming workshop organized

An online Brainstorming workshop on Seri-biotechnology was organized on 22nd December, 2021 with active participation of all Institutes of CSB conducting research in the area of Seri-biotechnology.

Workshops Attended

1. **Dr. Himanshu Dubey**, Scientist B attended online International Workshop on 'Basic to advanced data analysis in Genomics and Proteomics' organized by Nextgenhelper, New Delhi on 19th, 20th and 26th December 2020.
2. **Dr. Himanshu Dubey**, Scientist B attended online international workshop on "Genetic Variation Analysis Course" organized by C-CAMP and Swissnex India University of Zurich 9th-12th March 2021

राजभाषा कार्यान्वयन / OFFICAL LANGUAGE IMPLEMENTATION

राजभाषा कार्यान्वयन के तहत प्राप्त प्रगति इस प्रकार है:

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

- संस्थान ने अनुच्छेद 3(3) के प्रावधानों का अनुपालन किया है और सभी दस्तावेज द्विभाषी में जारी किए गए।

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

- संबंधित क्षेत्रों में हिंदी में पत्राचार के लक्ष्य (जोन ए में 100% और जोन सी में 55%) प्राप्त किए गए थे । इससे फाइलों में हिंदी टिप्पिणियों 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 2020 में SSTL और RSRS, के साथ मिलकर एक दिवसीय संयुक्त कार्यशाला का आयोजन किया गया।
  One joint workshop was organized in March 2020 jointly with SSTL and RSRS, Kodathi.
- हिंदी पखवाड़ा 1 से 14 सितंबर 2020 तक मनाया गया और विभिन्न कार्यक्रम आयोजित किए गए।
  Hindi Pakhwada was celebrated from 1st - 14th September 2020 by conducting various events.

PUBLICATIONS

1. Esvaran V, Jagadish A, Terenius O, Suraporn S, Mishra RK, Ponnuvel KM (2020) Targeting essential genes of *Nosema* for the diagnosis of pebrine disease in silkworms. **Annals of Parasitology**, 66(3):303-310.
2. Hassan W, Nath BS, Ponnuvel KM, Mishra RK, Pradeep AR (2020) Evolutionary Diversity in the Intracellular Microsporidian Parasite Nosema sp. Infecting Wild Silkworm Revealed by IGS Nucleotide Sequence Diversity. **Journal of Molecular Evolution**, 88(4):345-360.
3. Ito K, Ponnuvel KM, Kadono-Okuda K (2021) Host Response against Virus Infection in an Insect Bidensovirus Infection Effect on Silkworm (*Bombyx mori*). **Antioxidants** (Basel, Switzerland), 10(4).
4. Ramesha A, Himanshu Dubey, K. Vijayan, Ponnuvel KM, Mishra RK, Suresh K. (2020). Genome wide characterization revealed MnMLO2 and MnMLO6A as candidate genes involved in powdery mildew susceptibility in mulberry. **Molecular Biology Reports**, 2889-2900 doi: 10.1007/s11033-020-05395-6 PMID: 32239465
5. Sahar Ismail, Tulsi Naik, KS, Rajam, MV, Mishra RK (2020). Targeting genes involved in nucleopolyhedrovirus DNA multiplication through RNA interference technology to induce resistance against the virus in silkworms. **Molecular Biology Reports** 47, 5333–5342 doi: 10.1007/s11033-020-05615-z;
6. Shambhavi H. Prabhuling, Pooja Makwana, Pradeep AR, K. Vijayan and Mishra RK (2021) Release of mediator enzyme β- hexosaminidase and modulated gene expression accompany hemocyte degranulation in response to parasitism in the silkworm *Bombyx mori*. **Biochemical Genetics**. 59(4)997-1017.

Abstracts published in Workshops

1. Ramesha A, Himanshu Dubey, Vijayan K, Ponnuvel KM, Mishra RK, Suresh K. Targeting susceptibility genes for powdery mildew disease resistance in mulberry. International E-Conference on "**Multidisciplinary approaches for plant disease management in achieving sustainability in agriculture**" **6th - 9th October 2020**. (Oral presentation) organized by Department of Plant Pathology, College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot, India
2. Ramesha A, Himanshu Dubey, Vijayan K, Ponnuvel KM, Mishra RK, Suresh K. Genome wide characterization of MLO genes involved in powdery mildew disease susceptibility in mulberry. International E-Conference on "**Advances and Future Outlook in Biotechnology and Crop Improvement for Sustainable Productivity**" **24th to 27th November, 2020**. (Poster presentation) Organized by College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot, India.
3. Himanshu Dubey, Ramesha A, Ponnuvel KM & Mishra RK. Identification and in-silico expression analysis of NB-ARC domain containing genes in mulberry at International E-Conference on "**Multidisciplinary approaches for plant disease management in achieving sustainability in agriculture**" **6th-9th October 2020**. (Poster presentation) organized by Department of Plant Pathology, College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot, India

AWARDS

Young Scientist Award

1. **Dr. Himanshu Dubey**, Scientist B received NESA Young Scientist Award-2020 by the National Environmental Science Academy, New Delhi.

Paper / poster presentation

1. **Dr. Himanshu Dubey**, Scientist B received best poster presentation award for the poster entitled “Identification and in-silico expression analysis of NB-ARC domain containing genes in mulberry” at International E-Conference on Multidisciplinary approaches for plant disease management in achieving sustainability in agriculture” 6th - 9th October 2020 organized by Department of Plant Pathology, College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot, India

2. **Dr. Tulsi Naik**, Scientist C received the best paper presentation award with a certificate and cash prize of Rs 3000/- for the paper entitled “BmNPV late expression factors potent targets for inducing virus resistance against Grasserie infected *Bombyx mori* by RNA interference (RNAi) technology” at International conference on Biotechnology and Biological sciences-”Biospectrum” organized by Institute of Engineers Kolkata, West Bengal-INDIA

Ph.D awarded [University of Mysore]

1. **Ms. Shambhavi P. H.** (Guide: Dr. A. R. Pradeep, Scientist D)
   Thesis Title: Cellular and Molecular response of the Silkworm *Bombyx mori* to the microsporidian *Nosema bombycis* infection

RESEARCH ADVISORY COMMITTEE

S.No.	Details of Committee	Designation
1.	**Dr. N. K. Krishna Kumar** 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

S.No.	Details of Committee	Designation
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. Ashokan** Principal Scientist Dept. of Biotechnology, IIHR, Hesaraghatta Bangalore	DBT Nominee
5.	**Dr. H. K. Basavaraja** Retd. Scientist, Central Silk Board, Bangalore	Member
6.	**Dr. Raghunath,** MBBS Medical Officer, Kodathi, Govt. Medical Hospital, Sarjapura Road, Bangalore	Member
7.	**Dr. K. M. Ponnuvel** Scientist D, Seri-biotech Research Laboratory, Bangalore	Member-Internal Expert
8.	**Dr. A. R. Pradeep** Scientist D, Seri-biotech Research Laboratory, Bangalore	Member-Internal Expert

HUMAN RESOURCES

S.No.	Name	Designation
1	Dr. R. K. Mishra	Director
2	Dr. K. M. Ponnuvel	Scientist- D
3	Dr. A. R. Pradeep	Scientist- D
4	Dr. K. S. Tulsi Naik	Scientist- C
5	Dr A. Ramesha	Scientist- C
6	Dr. Himanshu Dubey	Scientist-B
7	Mr. R. N. Srikantaiah	Senior Technical Assistant
8	Mr. K. M. Humayun	Senior Technical Assistant
9	Mr. G. Raghavender	Field Assistant

RESEARCH FELLOWS / ASSISTANTS

S.No.	Name	Designation
1	Mr. Wazid Hussain	Research Associate (CSIR)
2	Ms. Shambhavi P Hungund	Junior Research Fellow
3	Ms. Dyna Susan Thomas	Junior Research Fellow
4	Ms. Chitra Manoharan	Junior Research Fellow
5	Ms. Sandhya Rasalkar	Junior Research Fellow
6	Ms. Merin Rose	Junior Research Fellow
7	Ms. Indumathi Kamatchi	Junior Research Fellow
8	Ms. Aneesha P.J.	Junior Research Fellow
9	Ms. Deeksha Khajje	Project Assistant
10	Ms. Vanitha C.	Project Assistant

ADMINISTRATION

S.No.	Name	Designation
1	Mr. R. Ranganath	Assistant Director
2	Ms. Manjula S.	Superintendent
3	Mr. Chandrashekhar Rao	Assistant Superintendent
4	Mr. Kenchappa	Multi tasking Staff

FINANCIAL PROGRESS

During the year under report, an expenditure of Rs. 240.06 lakhs was incurred under following heads from Grants-in-Aid sanctioned by CSB as well as funds received from external funding agencies.

S.No.	Particulars		Amount (Rs. in lakhs)
1	Salary and allowances	PLS	150.53
		SCS	32.64
		STS	13.85
2	PLG		20.00
3	Assets (PLC)		9.74
	Subtotal		**226.76**
4	External fund assistance		13.30
	Total		**240.06**

OTHER ACTIVITIES

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

Blank