वार्षिक प्रतिवेदन
Annual Report
2018-19

रेशम जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला
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Cover Page: Whole genome of muga silkworm *Antheraea assamensis* is ~500 Mb and *de novo* genome assembly made. The figure depicts gene ontology showing putative functional role associated with genes predicted from whole genome data of *A. assamensis.*

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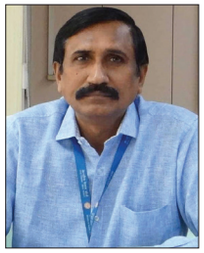
CONTENTS

प्रस्तावना



रेजैप्रौअप्र में किए गए शोध कार्य के मुख्य आकर्षण दर्शाते हैं कि उद्योग उन्मुख अनुसंधान ने पिछले साल पारंपरिक प्रजनन और रेशम–जैव प्रौद्योगिकी उपकरणों के सम्मिश्रण का उपयोग किया है । डीएनवी – प्रतिरोधी नस्लों और संयुक्त एनपीवी–डीएनवी प्रतिरोधी एमएएसएन नस्लों की पहचान ने शहतूत रेशमकीट जननद्रव्य समूह में विषाणु सहिष्णु प्रजाति की विद्यमानता दर्शाई । उल्लेखनीय रूप से इन जातियों के द्विप्रज संकर और बहु ङ द्वि संकर का क्षेत्र मूल्यांकन हुआ जिससे उत्तर भारत और पश्चिम बंगाल में मौजूदा प्रचलित नस्लों की तुलना में उपज और उत्तरजीविता में वृद्धि देखी गई ।

बी.मोरी में ट्रांसकिंगडम आरएनए हस्तक्षेप अभिगम का उपयोग कर एनपीवी प्रगुणन में ५०% की कमी प्राप्त की जा सकी । दूसरी ओर एनपीवी के समान विषाणु के कणों को विषाणु जीनोम अनुक्रम विश्लेषण के माध्यम से ओक तसर रेशमकीट एंथेरिया प्रायली में टाइगर बैंड रोग के प्रति जिम्मेदार रोगजनकों के रूप में पहचान की गई ।

सहयोगी कार्यक्रमों के माध्यम से एक और साधन संपन्न उपलब्धि मूगा रेशम कीट एंथेरिया असामेनसिस के पूरे जीनोम को अनुक्रमित और प्रतिलेखीय विश्लेषण करना था जिससे यह प्रदर्शित हुआ कि मूगा रेशमकीट जीनोम का आकार लगभग ५०० एमबी है जिससे विभिन्न शारीरिकीय भूमिकाओं से जुड़ी टिप्पणी के साथ लगभग २३००० जीन समाविष्ट थे । जीनोम आंकड़ा उत्पादन लक्षणों और रोग सहिष्णुता से संबद्ध आण्विक मार्करों के संवर्धन के प्रति उपयोगी हो सकता है, जिसे अधिक उत्पादन क्षमता और उत्तरजीविता के साथ नये मूगा रेशम कीट नस्लों को संश्लेषित करने में इस्तेमाल किया जा सकता है ।

प्रोटीन विलयन अभिगम के माध्यम से, फाइब्रॉइन–सेक्रोपिन संलयन प्रोटीन को संश्लेषित किया गया जो जैव चिकित्सा अनुप्रयोगों में उपयोगी हो सकता है । अति–अभिव्यक्त रेलिश और ड्रोसोमाइसिन जीन के साथ ट्रांसजेनिक रेशम कीटों ने माइक्रोस्पोरिडिया प्रबलता के लिए बेहतर सहिष्णुता दिखाई ।

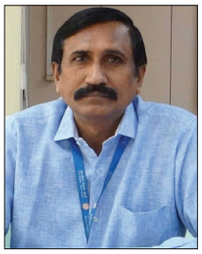
वैज्ञानिकों, अनुसंधान अध्येताओं एवं शोध छात्रों, तकनीकी और प्रशासनिक कर्मचारियों और सहायक कार्मिकों की टीम भावना के चलते ही यह उपलब्धि हासिल हो सकी । मैं इस अवसर पर उन शोध छात्रों की सराहना करता हूं, जिन्हें मैसूर विश्वविद्यालय से पीएचडी उपाधि से सम्मानित किया गया और नए रेशमकीट आण्विक जीव विज्ञान अनुसंधान में नए अनुसंधानों के लिए हमारे साथ जुड़े नए अन्वेषकों की भी प्रशंसा करता हूँ ।

केंद्रीय रेशम बोर्ड के निरंतर समर्थन, जैव प्रौद्योगिकी विभाग (भारत सरकार) और इंडो–स्वीडिश अनुसंधान कार्यक्रम से वित्तीय सहायता और अनुसंधान सलाहकार समिति एवं डीबीटी विशेषज्ञ समिति की तकनीकी सलाह के बिना यह प्रस्तावना अधूरी रहेगी । यह प्रयोगशाला, केंद्रीय रेशम बोर्ड के अधीनस्थ संस्थानों और राष्ट्रीय एवं अंतर्राष्ट्रीय प्रयोगशालाओं जैसे आईआईएससी, बेंगलूरु, आईआईटी गुवाहाटी से प्राप्त तकनीकी सहायता की ऋणी है । रेशम जैव प्रौद्योगिकी अनुसंधान कार्यक्रम को बेहतर बनाने के लिए विभिन्न संस्थानों से प्राप्त समर्थन के प्रति धन्यवाद देते हुए भविष्य में भी इन्हीं प्रयासों की अपेक्षा करता हूँ ताकि रेशम उद्योग लाभान्वित हो सके ।

डॉ. आर. के. मिश्र

निदेशक

Foreword



Highlights of the research work carried out at SBRL showed that industry - oriented research has taken lead in the past year utilizing an amalgamation of conventional breeding and Seri-biotechnology tools. Identification of DNV- resistant breeds and combined NPV-DNV resistant MASN breeds have shown the existence of virus tolerant races in the mulberry silkworm germplasm pool. Notably bivoltine hybrids and multi x bi hybrids of these races are under field evaluation which further showed enhanced yield and survival in comparison to existing ruling breeds in north India and West Bengal.

Reduction in NPV multiplication by 50% using Transkingdom RNA interference approach could be achieved in *B. mori*. On the other hand virus particles similar to NPV were identified as the pathogens responsible for Tiger band disease in the oak tasar silkworm, *Antheraea proyeli* through virus genome sequence analyses.

Another resourceful achievement through a collaborative program is the whole genome sequencing and transcriptome analysis of muga silkworm, *Antheraea assamensis* which showed size of the muga silkworm genome as approximately 500Mb comprising nearly 23000 genes associated with different physiological roles. The genome data could be useful to exploit the molecular markers associated with yield traits and disease tolerance which in turn can be used to synthesize new muga silkworm breeds with larger yield potential and survival.

Through fusion protein approach, fibroin-cecropin fused protein was synthesized which could be useful in biomedical applications. Transgenic silkworms with overexpressed Relish and Drosomycin genes showed better tolerance to microsporidia attack.

The achievements accomplished are outcome of the sincere team work of the scientists, research fellows and scholars, technical and administrative staff and supporting system. I take this opportunity to appreciate the research scholars who were awarded with the Ph. D Degree from University of Mysore and the new investigators who joined us to take new path in silkworm molecular biology research.

This foreword will be incomplete without mentioning the constant support from Central Silk Board, financial support from Department of Biotechnology (Government of India) and Indo-Swedish Research program and for the technical advises from Research Advisory Committee and DBT expert committee. This laboratory is indebted for the technical support received from National and International Laboratories like IISc, Bangalore, IIT Guwahati, University of Delhi (south campus) and sister Institutes under CSB. While thanking for the support received from different Institutions, the same is expected for our future endeavour to improve the Seri-biotechnology research programme to benefit the sericulture industry.

Dr. Rakesh Kumar Mishra
Director

VISION AND MISSION

VISION

To become a Centre of Excellence in Seri-biotechnology.

MISSION

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

MANDATE

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

OBJECTIVES

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

FUNCTIONS

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

अनुसंधान परिणाम की मुख्य विशेषताएं

१. प्रतिरक्षा जीन (रेलिश और ड्रोसोमाइसिन) की अभिव्यक्ति पर पारजीवी रेशमकीटों का सफलतापूर्वक विकास किया गया और इससे एनपीवी, नोसेमा बॉम्बिसिस और जीवाणु रोगजनकों के प्रति सहिष्णुता देखे गई ।

२. फाइब्रॉइन–सेक्रोपिन बी संलयन प्रोटीन की पुनर्योजन अभिव्यक्ति, विषम अभिव्यक्ति प्रणालियों में प्राप्त की गयी ।

३. जैव–आमापन के माध्यम से बीएमडीएनवी–२ प्रतिरोध के लिए सीएसआर 6–आर, सीएसआर२६–आर, एमएएससएन–6 आर, एमएएसएन 7 आर और जे२पी–आर के एनएसडी–2 मार्कर सहयोगी के साथ चयनित वंशक्रम का विकास और इन्हें मानकीकृत किया गया । सम–विषम स्थिति में एमएसडी–2 प्रतिरोधी विकल्पी वाले नर–मादा संकरण से उत्पन्न संकर पूरी तरह से बीएमडीएनवी–2 के प्रतिरोधी थे । वैसे, बीएमडीएनवी–2 प्रतिरोधी और उत्पादक रेशमकीट नस्लों एवं संकरों के विकास हेतु एनएसडी–2 लोकस पर चयन पर्याप्त है ।

४. डीएसआरएनए उपचारित एनपीवी संक्रमित रेशमकीटों के मध्यांत्र नमूनों के वास्तविक समय में मात्रात्मक विश्लेषण से ट्रांसकिंगडम आरएनए विश्लेषण से बीएमएनपीवी संक्रमित नमूने की तुलना में विषाणु जीन की संख्या में कमी देखी गई । जैव आमापन ने विषाणु प्रगुणन पर डीएसआरएनए के प्रभाव से बीएमएनपीवी संक्रमित रेशमकीटों के डीएसआरएनए उपचार के बाद उत्तरजीविता प्रतिशतता में > 50% वृद्धि हुई ।

५. तंतु लंबाई के लिए आनुवंशिक मार्कर [जीएफएल१] को 600 बीपी विकल्पी वाले बहुप्रज प्रजातियों और 700 बीपी विकल्पी वाले द्विप्रज प्रजातियों को पहचाना गया है और बी.मोरी के गुणसूत्र 16 में मानचित्रित किये गये हैं । वंशानुगत विश्लेषण से पता चला है कि पीएम ङ सीएसआर 2 और निस्तारी ङ सीएसआर 2 के एफ 1 में विषमयुग्मजी स्थिति में थे तथापि 1: 2: 1 दर से एफ 2 पीढ़ी में पृथक्करण देखा गया । रेशम ग्रंथि क्षेत्र के विश्लेषण से पता चला है कि दोनों विकल्पी (600 बीपी और 700 बीपी) वाले कीटों में एफआईबी–एल 2 ओर एफआईबी–एच, पी 25 के अभिव्यक्ति स्तर के 600 बीपी विकल्पी वाले कीटों की तुलना में अधिक पाया गया है जो तंतु लंबाई के साथ जीएफएल–1 के सहयोग को दर्शाता है ।

६. संक्रमित ओक तसर (ए.प्रायली) रेशम कीटों के विषाणु कण शुद्ध किए गये । पृथक और संरक्षित क्षेत्रों का जीनोमिक डीएनए कृंतक [क्लोन] कर इन्हें अनुक्रमित किया गया । विषाणु की पूर्ण लंबाई वाले जीनोम की पहचान की गई (अभिगम: जीआई : 1371952746)

७. एंथेरिया माइलिट्टा को संक्रमित करने वाले इफ्ला विषाणु को लक्षण निर्धारण किया गया । विभिन्न ऊतकों में विषाणु के प्रगुणन के साथ–साथ संचरण की विधि का विश्लेषण किया गया । मातृशलभ से संतानों में अंडों के माध्यम से अनुलंब संचरण देखा गया ।

८. एनपीवी सहिष्णु उच्च उत्पादन वाले द्विप्रज संकर, एमएएसएन 4 स्र सीएसआर 4 के क्षेत्र मूल्यांकन ने पूर्वी भारत में नियंत्रण (एसके 6 स्र एसके 7) में 50 किग्रा/100 रोमुच के सापेक्ष 52 किग्रा/100 रोमुच का कोसा उत्पादन दर्शाया । इसी प्रकार उत्तर–पूर्वी क्षेत्र में हिमाचल प्रदेश में ५१–५४ किग्रा /100 रोमुच और जम्मू व कश्मीर में 49 किग्रा /100 रोमुच की दर पर कोसा उत्पादन हुआ । मूल्यांकन अध्ययनों ने एमएएसएन वंशक्रम और संकरों में एनपीवी सहिष्णुता के लिए मार्करों की उपस्थिति की पुष्टि की ।

९. पूरे संजीन अनुक्रमण द्वारा मूगा रेशमकीट संजीन आकार लगभग 500 एमबी पाया गया । औसत समीपस्थ [कांटिग] लंबाई 185827.3 थी जबकि कुल समीपस्थ [कांटिग] की लंबाई 501176205 थी । पूरे कीटपालन संजीन से कुल 23,770 जीन और वन्य प्ररूप से 23,651 प्रोटीन का पूर्वानुमान किया गया । कीटपालन प्रजाति से कुल 17,527 प्रोटीन प्राप्त किया गया और वन्य प्ररूप से 17,415 प्रोटीन प्राप्त हुआ । संजीन के एसएसआर विश्लेषण से पेंटान्यू क्लयोटाइड्स और टेट्रा न्यूक्लियोटाइड को सम्मिलित करते हुए औसतन 25,600 एसएसआर प्राप्त हुआ ।

RESEARCH HIGHLIGHTS

1. Transgenic silkworms over-expressing immunity genes (Relish and Drosomycin) were successfully developed and have shown enhanced tolerance to NPV, *Nosema bombycis* and bacterial pathogens.

2. Recombinant expression and purification of Fibroin-Cecropin B fusion protein was achieved in heterologous expression systems for biomedical applications.

3. Developed and validated nsd-2 marker- assisted selection lines of CSR6-R, CSR26-R, MASN-6R, MASN7R and J2P-R for resistance to BmDNV-2 through bioassay. Hybrids generated by crossing parents carrying nsd-2 resistant allele in homozygous condition were completely resistant to BmDNV-2. Moreover selection at nsd-2 locus is sufficient for the development of BmDNV-2 resistant and productive silkworm breeds and hybrids.

4. Transkingdom RNAi studies found that the midgut samples of dsRNA-treated BmNPV- infected silkworms showed decrease in the copy number of viral genes compared to control samples. Bioassay validated the effect of dsRNA on viral multiplication and found that there was >50% increase in the survival percent after dsRNA treatment of BmNPV infected silkworms.

5. Genetic marker for filament length (GFL1) has been identified in multivoltine races with 600bp allele and in bivoltine races with 700 bp allele and are mapped Chromosome 16 of *B. mori.* Analysis of posterior silk gland region showed that in the individuals possessing both the alleles (600bp and 700bp) the expression levels of Fib-L and Fib-H and p25 were higher compared to individuals with 600bp allele only indicating association of GFL-1 with the filament length.

6. Virus particle in Tiger band disease infected oak tasar *(Antheraea proylei)* silkworms was purified. Genomic DNA isolated from the virus and conserved regions have been cloned and sequenced. The full length genome of virus revealed that the *Antheraea proylei* nucleopolyhedrovirus as a causative agent (Accession: GI: 1371952746).

7. Iflavirus infecting the *Antheraea mylitta* has been characterized. The multiplication of virus in different tissues as well as mode of transmission have been analysed. The vertical transmission through egg was observed from mother moth to the offsprings.

8. Field evaluation of NPV tolerant high yielding bivoltine hybrid, MASN4 x CSR4 indicated cocoon yield of 52 kg/100 dfls against 50 kg/100 dfls in the control (SK6 x SK7) in Eastern India. Similarly, in North-west region, the cocoon yield was in the range of 51-54 kg/100 dfls in Himachal Pradesh and 49 kg/100 dfls in Jammu. Validation studies confirmed presence of the markers for NPV tolerance in the MASN lines and hybrids.

9. Muga silkworm genome size is found approximately 500Mb by whole genome sequencing. Average contig length was 185827.3 whereas total contig length was 501176205. Total 23770 genes were predicted from whole genome of reared and 23651 from wild type muga silkworm. Total 17527 proteins were annotated from reared race and 17415 proteins annotated from wild types. SSR analysis of the genome showed an average number of 25600 SSRs including penta-nucleotides and tetra-nucleotide repeats.

LIST OF RESEARCH PROJECTS

ONGOING RESEARCH PROJECTS

1. **AIT 3538:** Development of Fibroin Fusion Silk with Antioxidant and Antibacterial Properties (May 2015- 2019)

2. **AIT 3583:** Transkingdom RNA interference approach for disease resistance against BmNPV infection in silkworm *Bombyx mori* - Collaboration with University of Delhi, South Campus (Sep 2016-Aug 2019)

3. **AIT 3584:** Identification of molecular markers associated with filament characters and its use in improvement of multivoltine races- Collaboration with CSR&TI, Mysore and CSTRI, Bangalore (Sep 2016-Aug 2019)

4. **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 (funded by Department of Biotechnology, Govt of India, New Delhi)- Network project with CSR&TI, Mysore, CSR&TI, Berhampore and CSR&TI, Pampore (Feb 2017 – Jan 2020)

5. **ARP-3606:** Development of diagnostic tool for early detection of baculovirus causing tiger band disease in *Antheraea proylei* (Feb 2017 – Jan 2020)

6. **ARP- 08001 CI :** Studies on the genetic characterization, transmission and tissue distribution of Iflavirus infecting the Indian tropical tasar silkworm, *Antheraea mylitta* (Funded by Swedish University of Agricultural Sciences, Sweden)

CONCLUDED RESEARCH PROJECT

1. **AIT 3540:** Development of Transgenic Silkworms for the Over-expression of Disease-Resistant Genes for Enhanced Immunity- Collaboration with IISc, Bangalore (July 2015- March 2019)

2. **AIT 3582:** Development of densovirus resistant productive bivoltine silkworm breeds through marker assisted selection - Collaboration with CSR&TI, Mysore (Sep 2016-Aug 2018)

3. **AIT 5872:** Whole Genome Sequencing and functional genomics of Golden Silk Moth, *Antheraea assamensis*- Collaboration with IISc, Bangalore, IIT, Guwahati, CDFD, Hyderabad, CMERTI, Lahdoigarh (Oct 2015 –Mar 2019)

PROGRESS OF ONGOING RESEARCH PROJECTS

AIT 3538: Development of Fibroin Fusion Silk with Antioxidant and Antibacterial Properties

G. Ravikumar, K. Vijayan*,
M. Chitra and Dyna Susan Thomas, JRFs
*CSB, Bangalore

Objective:

To make a multifunctional silk fusion protein

Silk proteins from silkworm, *B. mori* are now recognized as excellent biomaterials for biomedical applications. The aim of the project is thus to develop a recombinant fusion protein of fibroin-cecropin B for wound healing and cell culture applications. Fibroin-Cecropin B was expressed, detected and purified from *Pichia pastoris*. To get a better yield of the recombinant fusion protein for various assays, new *Pichia* clones were tested for their expression levels. Out of ten clones, two clones were short-listed for further assays. These clones were used for scaling up the expression in large volumes using shake flask culture. SDS-PAGE and Western Blot detected the recombinant proteins from supernatant and cells. As observed earlier, more amount of recombinant proteins were confined to supernatants.

The vector construction with fibroin promoter and fibroin – cecropin fusion gene was completed for the expression of fusion protein in the cocoon. Vector construct consisted of fibroin heavy chain promoter followed by Exon 1, Intron 1 and 5' Exon 2, fusion gene, 3' Exon 2 along with polyA tail (Fig. 1). In addition GFP gene with ELF promoter is used as screening marker for the transgene. The construct is introduced into the piggyBac vector and microinjected to pre-blastoderm eggs for germline transformation. Transgenic silkworms showed the presence of GFP in the cocoons indicating the successful expression of transgene.

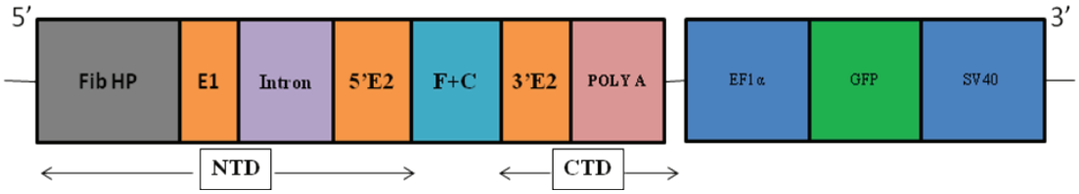


Fig. 1: Vector construction for the Fibroin-Cecropin fusion expression in cocoon by transgenesis

Fig. 2: Cocoons of transgenic silkworms showing GFP expression in CSR2 race

AIT 3583: Transkingdom RNA interference (tkRNAi) approach for resistance against BmNPV infection in silkworm *Bombyx mori.* L

K. S. Tulsi Naik, K. M. Ponnuvel, and M.V. Rajam*
Ms. Sahar Ismail , JRF
*Dept. of Genetics, University of Delhi, Delhi

Objectives

a) To identify and analyze the essential viral gene(s) involved in BmNPV multiplication.
b) To clone the essential viral genes in suitable vector that can express dsRNA.
c) To validate the effect of dsRNA on viral multiplication.
d) To analyze the viral copy number in dsRNA treated silkworms as well as control silkworms infected with BmNPV

To check the effect of dsRNA against the viral genes, the mid gut tissue from the dsRNA fed silkworms was dissected after 24 hrs and the genomic DNA was analyzed for the viral copy number using Gp41 primer. The real time PCR analysis of the genomic DNA showed approx. $1x10^7$ copies of virus in the NPV infected and NPV+L4440 fed silkworms, but the copy number was significantly low 100 copies in the dsRNA fed infected silkworms. This result clearly indicate that the dsRNA ie-1 and dsRNA lef-1 fed silkworms showed the RNAi effect and inhibited BmNPV multiplication (Fig 3A). Further, the study was extended to compare the differential expression of viral genes ie-1, lef-1 and Gp41 in the NPV infected and dsRNA fed infected silkworms by real-time analysis after 24 hrs of feeding. The NPV infected silkworms fed with dsRNA ie-1and dsRNA lef-1 constructs showed decrease in the transcript levels of GP41, ie-1 and also lef-1.

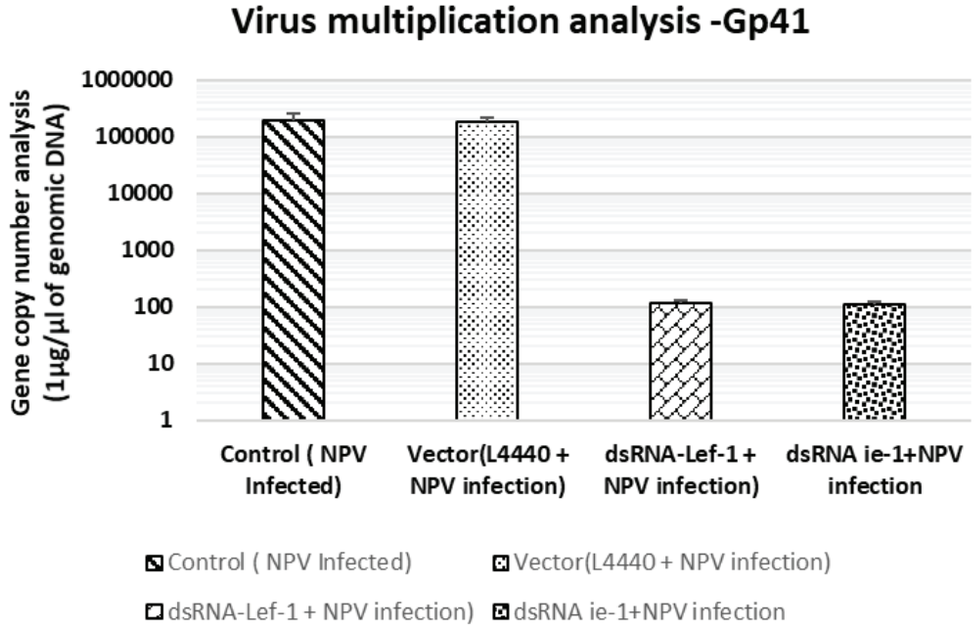
Among both the constructs that were fed independently to the infected silkworms, the dsRNA ie-1 construct was more effective and also the mRNA levels of lef-1 gene ie-1 genes werel comparatively lower than the mRNA levels expressed in dsRNA lef-1 constructs, this may be attributed to the fact that early genes ie., Ie-1 genes are generally encoded proteins of regulatory functions and modifications of host process and generally the early gene transcription takes place within 0-6hpi and late and very late transcription is dependent on early viral gene expression and viral DNA replication (Asha et al., 2002, Katsuma et al., 2007) (Fig 3B). Therefore the study showed that dsRNA fed silkworms increased the tolerance level to the virus infection by producing siRNA specific to viral mRNA which in turn elicit RNAi effect against the viral genes in *Bombyx mori* and due to this the virus particles were unable to synthesize proteins that were required for its multiplication and formation of virus particles.

Based on the above results, the survivability analysis was done for three batches to check the effect of dsRNA against the infected larva, the number of dead larvae against the survived larvae was calculated for different treatments ie, dsRNA ie-1 and dsRNA lef-1after BmNPV infection till the cocoon formation. In larvae fed with NPV and NPV + L4440 vector alone 1-2% of the larvae looked healthy and spun cocoons and the remaining larva died due to infection. The Ie-1 dsRNA fed BmNPV infected larvae showed 52.8% survival against the infected silkworms and Lef-1dsRNA fed BmNPV infected larvae showed 58.8% survival rate (Fig. 4). The overall results indicated

>50% increase in the survivability of virus-infected silkworms fed with dsRNA lef-1 and dsRNA ie-1 compared to infected silkworms. The survivability may be attributed to the RNAi mechanism triggered by the siRNA in the midgut tissue upon virus infection.

This study has shown that feeding bacterially expressed dsRNA led to a significant drop in the transcript levels of viral genes and restricted the viral multiplication in the host as compared to control. Hence, bacteria expressing the desired dsRNA(s) against viral gene(s) in insects may be effectively used for combating viral infections in silkworm *Bombyx mori*

A.



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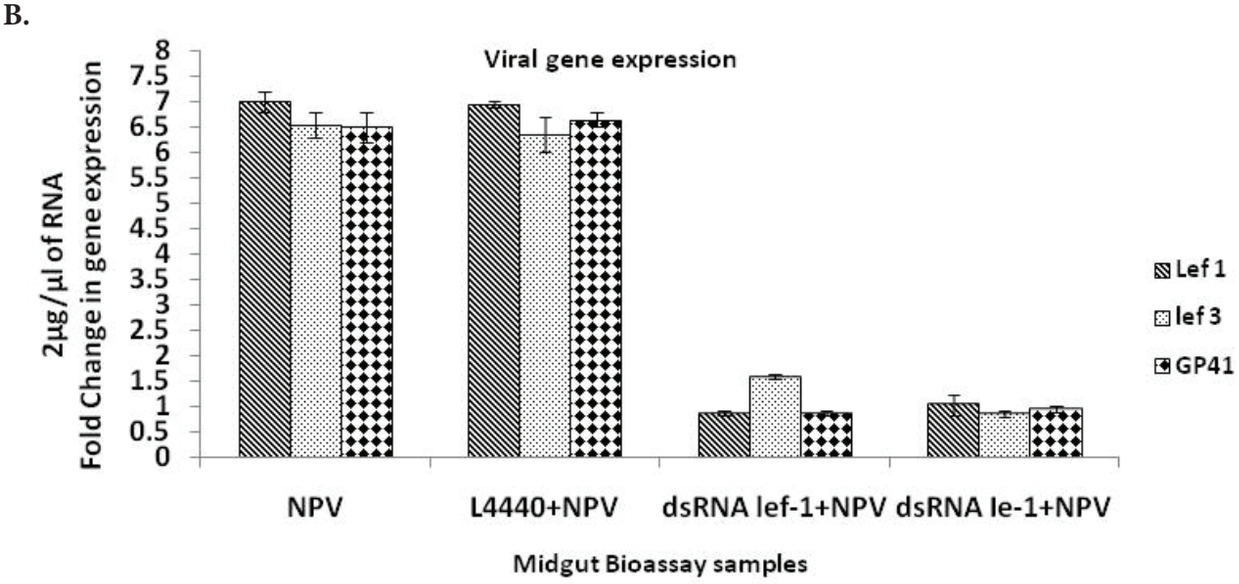


Fig. 3 : Expression analysis of genes involved in NPV multiplication: (A) Copy Number estimation using Gp41 specific primers in bioassay samples dissected from NPV infected and dsRNA fed infected silkworms (B). Quantitative analysis of BmNPV viral gene expression in midgut samples of dsRNA fed infected silkworms.

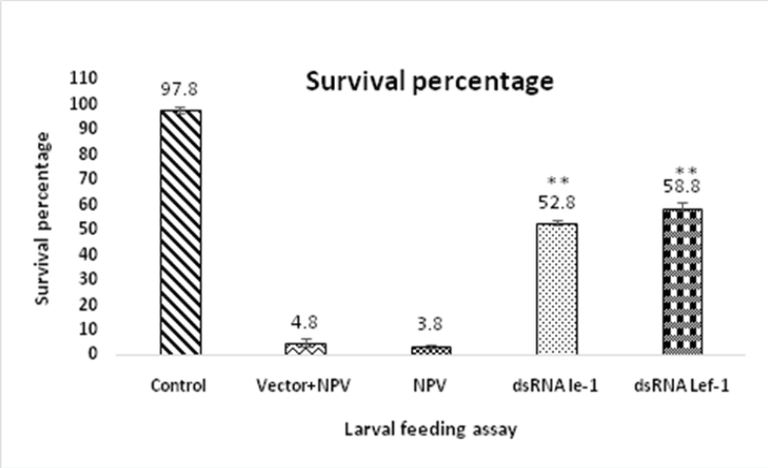


Fig. 4: Bioassay experiments to validate the effect of dsRNA lef-1 and dsRNA ie-1 in BmNPV infected silkworms.

AIT 3584: Identification of molecular markers associated with filament characters and its use in improvement of multivoltine races

(In collaboration with CSR&TI, Mysore and CSTRI, Bangalore)

K. S. Tulsi Naik and A.R. Pradeep
G. Hariraj* and L. Kusuma**
Ms. Sahar Ismail and Mr. S. Aravind JRFs,
*CSTRI, Bangalore and **CSR&TI, Mysore

Objectives

a) To identify and analyze association of selected candidate genes with the filament characters in the divergent multivoltine accessions

b) To develop F_2 & F_3 populations from divergent multivoltine parents for analyzing the segregation and inheritance pattern of markers associated with filament characters.

Multivoltine races are relatively inferior in terms of qualitative traits but superior in their survival and hardiness. Therefore, in this context development of cross breeds (M x B) has gained importance over the years owing to field constraints, fluctuations in the environmental conditions as well as socio economic compulsions and it is also strongly believed that the lustre, dye uptake and the stiffness of cross breed is superior compared to bivoltine silk and largely preferred by the reelers. The variations that exists between the bivoltine and the multivoltine races with respect to silk quality can be attributed to the differential expression of the major genes associated with silk proteins. Therefore, in this study real time PCR analysis was done to identify the genes that are differentially expressed in the posterior silk gland region (PSG) of the popular multivoltine races and was statistically correlated with genes involved in silk processing.

Posterior Silk gland (PSG) is the region where fibroin Heavy chain (Fib-H), Fibroin Light chain (Fib-L), p25, ion transport genes, cuticular proteins and other genes that are responsible for silk protein synthesis are produced. Among the multivoltine races, the expression level of Fib-L, Fib-H and p25 was significantly high at $p<0.05$ in Kollegal Jawan, Sarupat, Ap12, MW13 compared to other pure multivoltine races. The cross breeds PM x CSR2 and Nistari x CSR2 also showed significantly high expression of Fib-L, Fib-H and p25 compared to their multivoltine parents, but expression level did not reach to the level of bivoltine races any of the above genes. Other genes like ion transport genes, cuticular proteins, serine –glycine metabolism pathway genes showed significantly higher expression at $p<0.05$ in the cross breeds PM x CSR2 and Nistari x CSR2 compared to pure multivoltine races, these results indicated that though Fib-L, Fib-H and p25 are the major genes associated with silk filament and variations in gene expression was very clear among the races, but other genes that are responsible of ion transportation, maintain acidic environment and serine –glycine metabolism pathway genes are the ones that contribute to efficient silk processing and production (Fig 5).

The Pearson correlation also showed positive correlation existing between the genes that are taken in this study (Fig 6), indicating that with increase in expression of ion transportation genes, maintain acidic environment, cuticular genes and serine–glycine metabolism pathway genes the silk protein expression was significantly increased. It is also observed that these genes are significantly expressed at higher rate in the bivoltines, and that is the reason they are able to spin better quality silk compared multivoltine races. Hence, it is highly relevant to identify multivoltine germplasm accessions possessing high yield characters particularly filament characters which can be used to produce new silkworm breeds possessing better filament characters.

Post Cocoon analysis

The seven important characters/parameters, Shell ratio, Average filament length, Reelability, Renditta, Raw silk percent, cleanness and neatness were compared between the parents and the F_1 individuals (cross breeds). The cross breeds PM x CSR2 and Nistari x CSR2 showed significant increase in Shell ratio, Reelability, Raw silk percent, Cleanness and Neatness and significant decrease in the Renditta when compared with multivoltine parents. The Pearson correlation showed positive correlation existing between six characters, except Renditta which had a negative correlation with other six characters indicating that with decrese in Renditta there was a significant increase in Reelability, Raw silk percent, Cleanness and neatness (Fig 6). The quality of silk produced from theses cross breeds were highly improved and showed 2A gradable silk compared to their multivoltine parents most of the characters met the bench mark specified for cross breeds. The results clearly indicate the scope for developing potential cross breeds with improved yield characters, particularly filament characters to support the Indian sericulture industry.

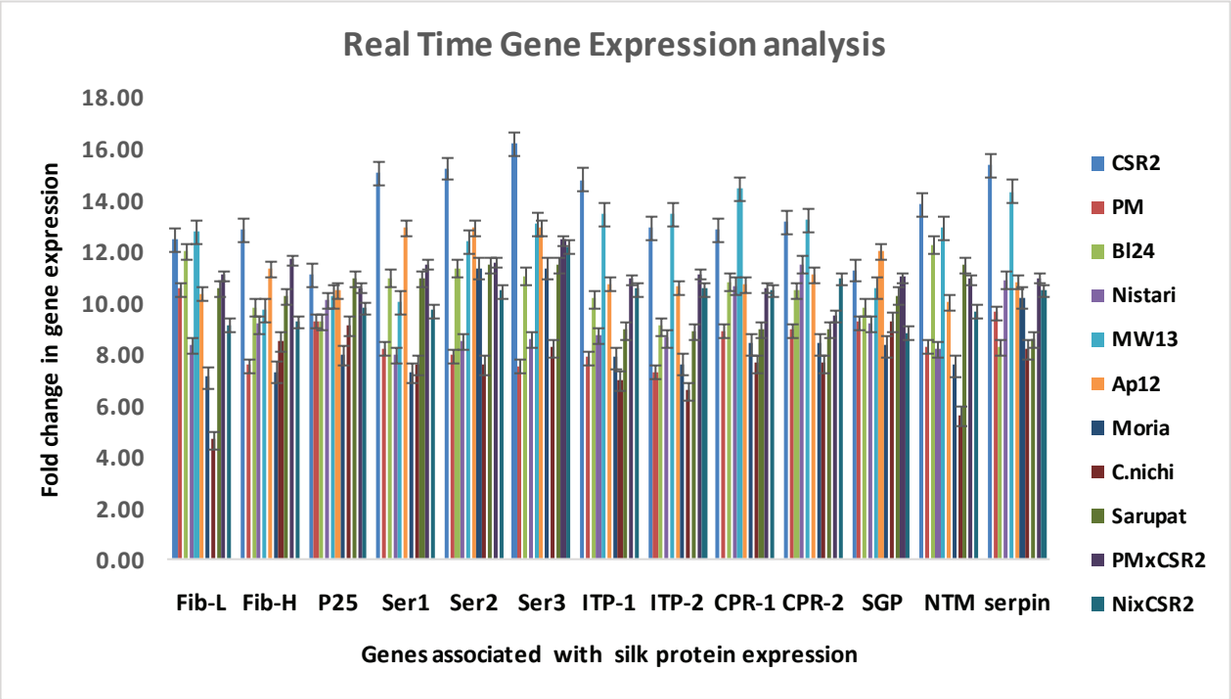
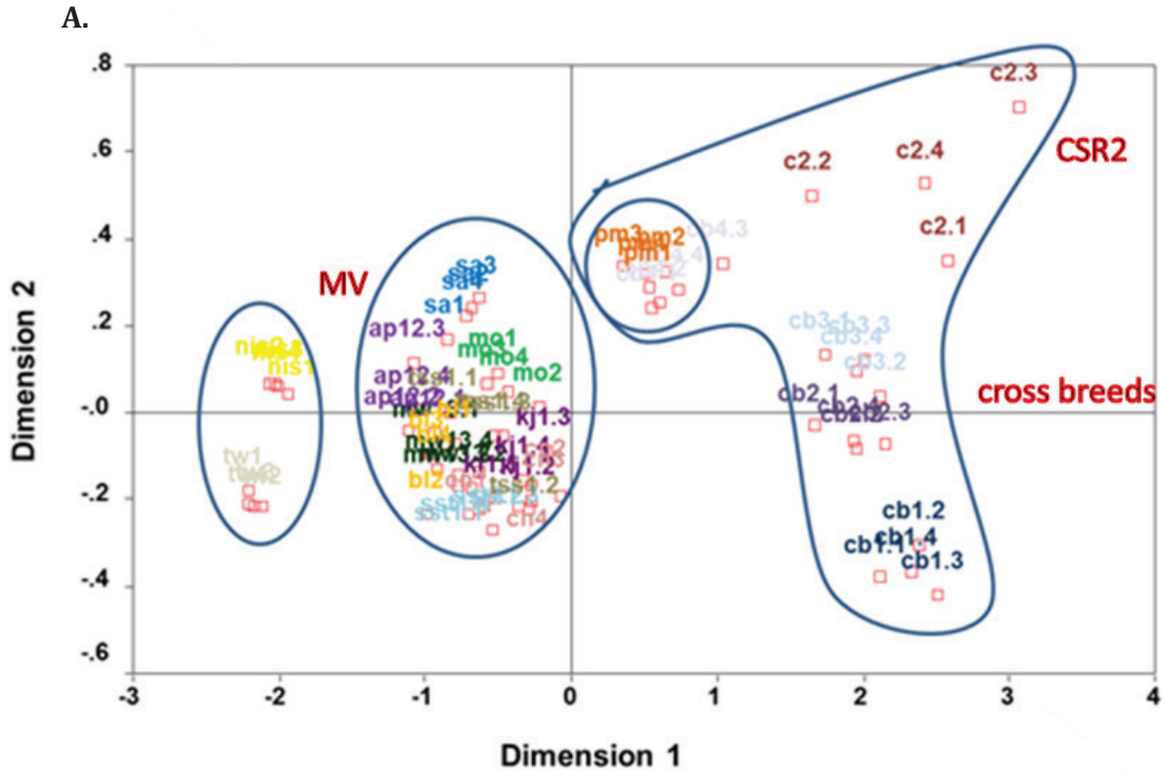


Fig 5: Differential gene expression analysis of genes associated with silk filament character using quantitative PCR



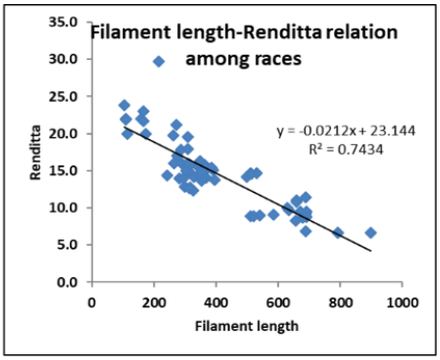
B.

Correlations

		SR	REEL	RS	RSR	WASTE	AFL	NBFL	SCFD	REND
SR	Pearson Co	1								
	Sig. (2-taile	.								
REEL	Pearson Co	**0.3175	1.000							
	Sig. (2-taile	0.008	.							
RS	Pearson Co	**0.8298	0.233	1.000						
	Sig. (2-taile	0.000	0.056	.						
RSR	Pearson Co	-0.092	-0.026	**0.460	1.000					
	Sig. (2-taile	0.456	0.830	0.000	.					
WASTE	Pearson Co	**-0.409	*-0.2823	**-0.666	**-0.60	1.000				
	Sig. (2-taile	0.001	0.020	0.000	0.000	.				
AFL	Pearson Co	**0.8361	**0.3197	**0.892	*0.3077	**-0.652	1.000			
	Sig. (2-taile	0.000	0.008	0.000	0.011	0.000	.			
NBFL	Pearson Co	**0.8174	**0.5500	**0.812	0.216	**-0.663	**0.952	1.000		
	Sig. (2-taile	0.000	0.000	0.000	0.076	0.000	0.000	.		
SCFD	Pearson Co	**0.5783	-0.152	**0.591	0.139	**-0.322	**0.643	**0.5585	1.000	
	Sig. (2-taile	0.000	0.215	0.000	0.257	0.007	0.000	0.000	.	
REND	Pearson Co	**-0.712	**-0.328	**-0.912	**-0.564	**0.6944	**-0.833	**-0.792	**-0.4964	1
	Sig. (2-taile	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).



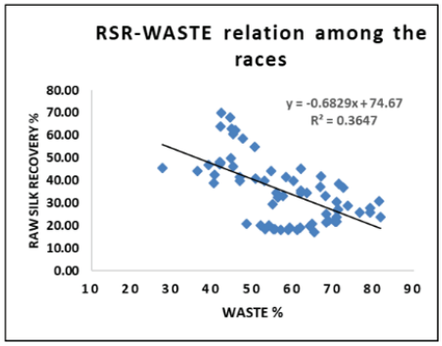


Fig.6: Multidimensional scaling (ALSCAL) (A) and Pearson correlation (B) of post cocoon parameters among multivoltine, bivoltine and the cross breeds.

ARP 3605 (DBT): 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&TI's of Mysore, Berhampore and Pampore and in technical collaboration with NSSO, Bangalore)

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

A. R. Pradeep, K.M. Ponnuvel and Gopika Padmakumar (JRF) SBRL, Bangalore

Dr. S. Manthira Moorthy [#] B. Mohan[##]

[#] CSR&TI, Mysore & [##]SSBS, Coonoor

Dr.Gopalchandra Das[1] and N. Chandrakanth

[1] CSR&TI, Berhampore

Sardar Singh[2] , Mohamed Aslam[2] and Pankaj Tiwari[3]

[2] CSR&TI-RSRS Jammu; [3] RSRS, Dehradun

Objectives of the project under CSR&TI Mysore, Berhampore and Pampore

a) To evaluate evolved lines in various agro-climatic conditions and select lines for their suitability in that particular environment at RSRS/ REC at Bangalore, Salem, Chamarajnagar, Ananthapur, Berhampore, Jammu Kashmir and Dehradun

b) To Prepare DFLs of PM / Nistari x MASN and MASN x CSR4 through NSSO (CSB) - Bangalore and distribution to Sericulture farmers for field evaluation. Few DFLs of CSR2 x CSR4 will also be produced for control observations.

Objectives under SBRL:

a) Validation of DNA markers for NPV resistance and stress tolerance in selected lines being used for field trials.

b) Continuous maintenance of MAS-N lines, Co-ordination and statistical analyses of observations from lines reared at different stations

Improvement of sericulture in the country depends on synthesizing new breeds with specific traits by crossing between multivoltine x bivoltine and bivoltine x bivoltine races. Marker Assisted Selection (MAS) lines for NPV tolerance and high yield traits were developed from Sarupat x CSR2 cross through selection processes. The lines were reared for over 45 generations and were used as parents for hybrid development by crossing Nistari / PM x MASN4 and MASN4 x CSR4 for field level test.

Performance of the cross with multivoltine and bivoltine races were assessed initially at SBRL and the successful crosses of MASN4 x CSR4 (bi x bi), Nistari x MASN4 (multi x bi) and PM x MASN4 (multi x bi) were used for field test. MASN4 x CSR4 (bi x bi) and Nistari x MASN4 (multi x bi) were tested at Berhampore sericulture districts and MASN4 x CSR4 (bi x bi) and Pure Mysore x MASN4 (multi x bi) were tested in South India. At Jammu and Dehradun, MASN4 x CSR4 (bi x bi) was tested for field trial.

Obj. 1: Validation of DNA markers for NPV resistance and stress tolerance in selected lines being used for field trials

The lines selected were evaluated for presence of DNA markers associated with NPV tolerance Viz. Three markers identified at SBRL, three genic markers developed from deoxy kinase (Nag 88), protein kinase G-1B (Nag 65) and glucosyl transferase (Nag 84).

MASN 4, 6 & 7 lines are reared and maintained at SBRL continuously and the presence of markers are confirmed. All the three lines are uniform in yield traits however, MASN4 showed better yield traits than other two lines. In addition to SBRL lines, MASN lines have been maintained at Silkworm Breeding station, Coonoor which is the source for parents to develop F_1 hybrids. As reported earlier, on an average of 90% moths showed presence of the markers indicating requirement of further stabilization of the lines through continuous screening and validation. The F_1 hybrids collected from Berhampore showed uniform amplification.

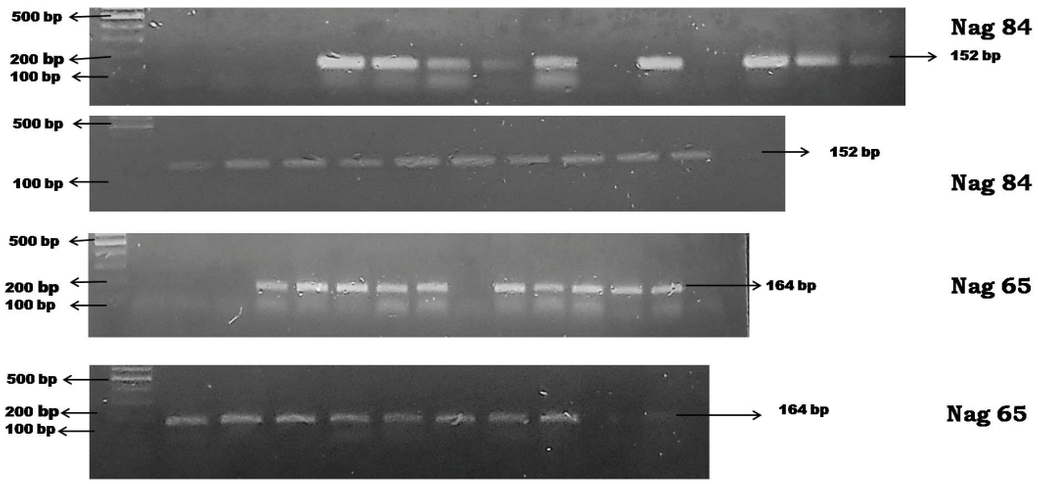


Fig 7: Amplification profile of F_1 individuals with different NPV tolerance associated markers showing uniform presence except absence in few individuals

Obj 2: Continuous maintenance of MAS-N Lines; Co-ordination and statistical analyses of observations from lines reared at different stations.

At SBRL, we maintain MASN4, 6 & 7 lines throughout the year. The DFLs are preserved for 4 months or 6 months period schedule in the cold room facility available with CSB-NSSO at Hosur or Mysore. After the preservation duration, the eggs will be released and reared and the cycle is repeated.

Original cross of Sarupat x CSR2 (parental cross of MASN) had silk ratio (SR%) of 20.88 ± 1.2% which was improved to 22.78 ± 1.08 % in back crosses (Sarupat x CSR2) x CSR2 and presently after 30 generations it has been stabilized at 20 ± 1.3%. SR% of the hybrid (MASN4 x CSR4) has reached upto 20.86 ± 3.83% (range 20 – 24%). However SR% of CSR2 x CSR4 has reached a higher peak of 23% though achieving this level is difficult as both CSR2 and CSR4 parents are highly susceptible to pathogens. Hence the MASN lines are predictably better in tolerance as well as carry stabilized high yield parameters.

Under summer conditions of Bangalore, high temperature combined with low humidity led to poor leaf quality and low moisture content. Under this condition, the MASN 4, 6 and 7 lines showed SR% of 19 – 20% there by showing traits in pure races. In addition to the MASN lines, the pure lines are used to develop cross/ hybrids of Nistari x MASN, PM x MASN and MASN4 x CSR4 (Fig. 8) which showed 95-99% survival in laboratory rearing conditions. These larvae showed better yield traits.

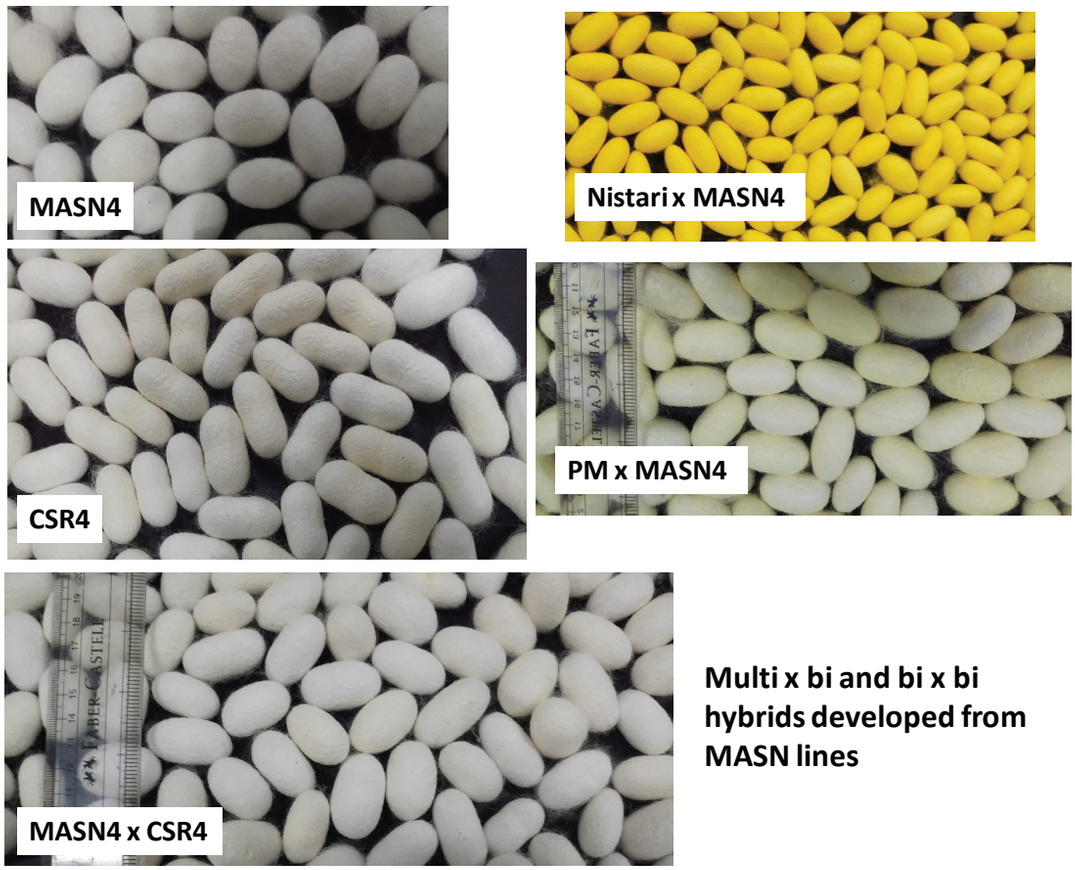


Fig 8: Cocoons developed from parents, multi x bi crosses and bi x bi hybrids from MASN4 parents.

The MASN x CSR4 produced a larger yield of 88.93 kg cocoons / 100 DFLs whereas the control CSR4 x CSR2 produced a higher yield of 95 Kg/ 100 DFL under our rearing conditions. However achieving the higher target is difficult because under field conditions, both parents CSR2 and CSR4 are highly susceptible to various diseas. On the otherhand MASN4 is a better tolerant breed and consistently better yield could be produced in the field.

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)

K. M. Ponnuvel & Diksha Khajje (JRF)

S. Subharani N* & Ibotombi Singh*

*RSRS, Imphal, Manipur

The oak tasar silkworm, *A.proylei* is a semi-domesticated sericigenous insect which is commercially exploited for the production of silk. Oak tasar silk production has declined in the recent past and the estimated silkworm crop loss is more than 60-80 %, mainly due to tiger band disease. The disease outbreaks are high during summer due to fluctuations in temperature and humidity. The disease could be transmitted through transovum/transovarial means and is an important issue in the management of the disease. Due to limited information available on tiger band disease such as causative agent , the spread of disease etc., it is imperative to develop precise diagnostic tools for the early detection as well as sources of infection to prevent (or) and to have a better control on the spread of the disease. The complete viral genome sequencing revealed that the group I alphabaculovirus i.e. *Antheraea proylei* nucleopolyhedrovirus (AnprNPV) is the casauitive agent of this disease

i. Characterization of baculovirus causing Tiger band disease in Oak tasar silkworm, *Antheraea proylei*

The Viral particles were isolated and purified to extract viral genomic DNA from infected oak tasar (*A. proylei*) silkworms (samples obtained from fields) for the purpose of characterization of the Baculovirus (Fig. 9). In support of characterisation studies, the viral conserved regions were targeted for designing primers to aid in further (cloning and sequencing) studies. The complete viral genome sequencing revealed that the group I alphabaculovirus i.e. *Antheraea proylei* nucleopolyhedrovirus (Anpr NPV) is the causative agent of this disease.

The full length genome of the virus has been sequenced (Accession: GI: 1371952746) and the analysis of the complete viral genome revealed six conserved regions. The conserved regions of *Antheraea proylei* nucleopolyhedrovirus (AnprNPV) were further found to share homology with *Antheraea pernyi* NPV (AnpeNPV)(Fig-10). Phylogenetic analysis of the viral genome revealed clustering of AnprNPV in one clade of alphabaculovirus group I of the saturniidae silkworm viz. *A. pernyi* and *Philosamia cynthia ricini,* indicating a close relationship between them. Furthermore, it was found that the isolates of AnprNPV and AnpeNPV had sequence identity above 95%, leading to a conclusion that AnprNPV may be a variant of AnpeNPV.

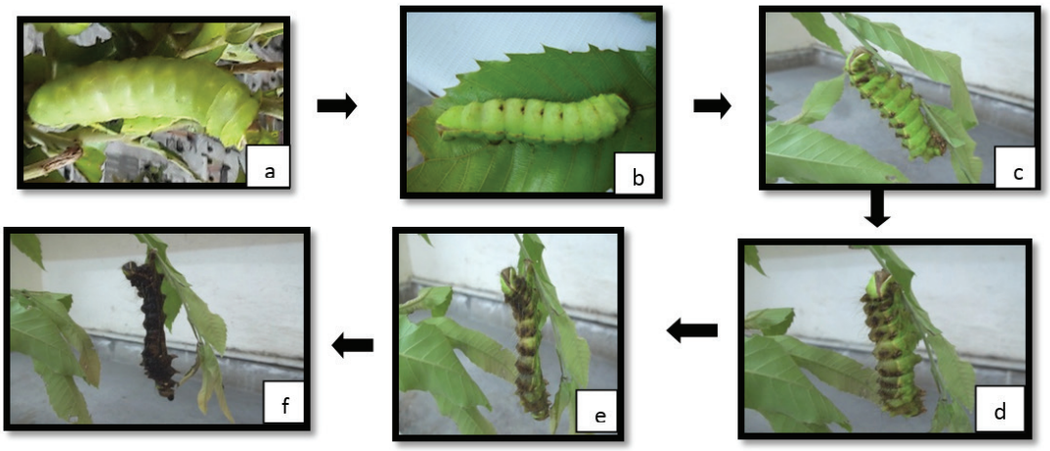


Fig. 9: Infected Oak Tasar Silkworm with disease manifestation of tiger band disease

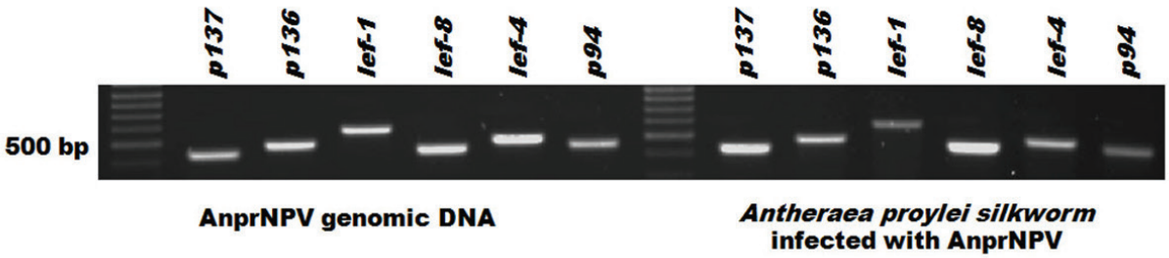


Fig. 10: The PCR amplification of AnprNPV using primers targeted for the conserved regions.

(i) Pathogenesis:

The mode of infection of the viral pathogen was studied initially by analysing the surfaces of the *A. proylei* eggs. The virus particles were detected on silkworm egg surfaces and also in its inner contents. The presence of viral particles was detected and confirmed on surfaces of both washed as well as unwashed eggs, through conventional PCR technique . In order to counter the issue of surface transmission of disease, appropriate egg disinfection method has been developed tried, tested and found effective on overcoming the spread of the disease.

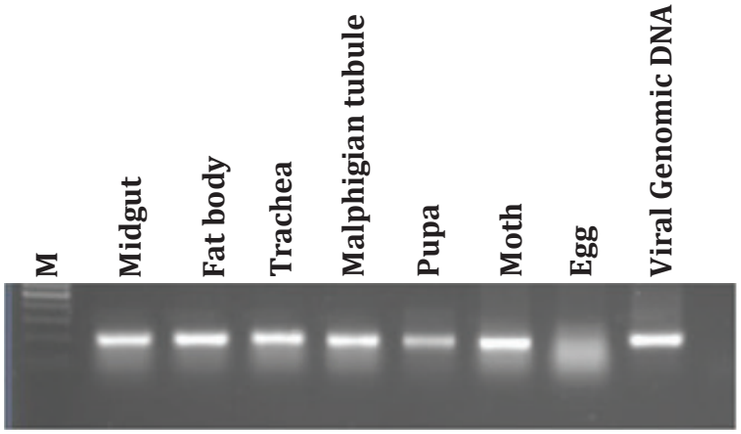


Fig. 11: The PCR amplification of AnprNPV using primers targeted for the conserved regions.

iii. Detection of disease at various life cycle stages of silkworm using PCR

The presence of virus particles was also detected at different developmental stages of the silkworm *A. proylei* . The PCR technique was successfully used to detect the virus at the egg, larval and moth stages. The viral copy number in the different tissue samples i.e egg, larva and moth were also quantified through qPCR.

The above analysis techniques have also led to the identification of other viral pathogens such as Iflavirus which can cause co-infection in oak tasar silkworms *Antherea proylei* along with baculovirus. The co-infection was detected using the primers specific for both the viruses (Baculovirus and Iflavirus). The p94 segment specific primer was used for the detection of AnprNPV(baculovirus) while the RdRp specific primer was used for the detection of ApIV(Iflavirus). Genomic DNA and cDNA obtained from infected *Antheraea proylei* moths were used as templates for the detection of AnprNPV and ApIV, respectively. The conventional as well as the Real time PCR, both showed amplification for AnprNPV and ApIV, respectively. These results clearly indicated that silkworms (*A. proylei*) can be co-infected by different viruses simultaneously. However, the impact of this co-infection needs to be analysed. Further, the detection of co-infection at different tissues and stages of infected *A. proylei* through Real time PCR was attempted. Different tissues from infected *A. proylei* samples were dissected, processed and tested for the presence of both AnprNPV and ApIV. The analysis revealed the presence of both the viruses in almost all the tissues and stages, which included the midgut, fat body, trachea, Malpighian tubule, ovary, pupa, moth and eggs (Fig 11). ApIV was detected in all the tissues and stages while AnprNPV was detected in all except the moth, pupa and egg stages.

(iv) Development of Diagnostic tool for early detection of the Tiger Band Disease

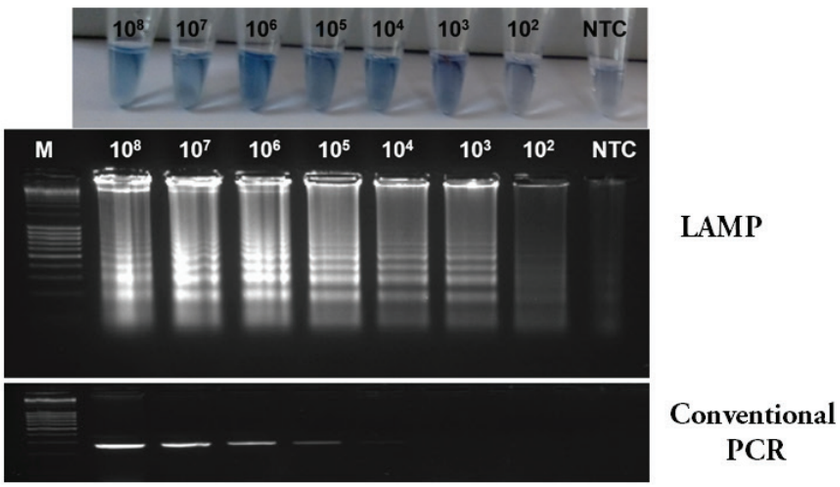


Fig 12: Comparison of Lamp Assay with conventional PCR using serially diluted p94 gene of AnprNPV cloned in pGMT-T vector.

A Loop-mediated isothermal amplification (LAMP) technique was standardized for easy & simple identification of the virus infection (Fig.12). The virus load was quantified in the different tissues and eggs of infected oak tasar silkworms. The genomic DNA from the infected samples was used as template for the LAMP assay. The technique proved to be simple convenient and allowed an early detection of infection.

ARP- 08001 CI: Studies on the genetic characterization, transmission and tissue distribution of Iflavirus infecting the Indian tropical tasar silkworm, *Antheraea mylitta* (Funded by Swedish University of Agricultural Sciences, Sweden)

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, Sweden

The tropical tasar silkworms, *Antheraea mylitta* and *Antheraea proylei* are affected with gastrointestinal disease and the causative agent is suspected to be a virus. EST database developed by various investigators in *A. mylitta* included a transcript that was found to have strong similarity with that of Iflaviruses, a genus of positive sense single-stranded RNA viruses belonging to the order Picornavirales, which are primarily infecting insects. In this study the full length genome sequencing of this novel virus was attempted.

The genome organization, amino acid sequence similarity and phylogenetic analyses all indicate that the virus is a new member of the genus Iflavirus - closely related to *Antheraea pernyi* Iflavirus (91% amino acid similarity), with the proposed name of *Antheraea mylitta* Iflavirus (AmIV). The genome sequencing revealed 9837 nucleotides of contiguous sequence excluding the natural poly-A tail, encompassing the full length of the single, large open reading frame, flanked by untranslated regions at 5′ and 3′ ends. As per the available literature, open reading frame is predicted to encode a 2967 amino acid polyprotein possessing four viral structural proteins (VP1-VP4) located at the N-terminal end and the non-structural proteins, including a helicase, RNA-dependent RNA polymerase and 3C-protease, located at the C-terminal end of the polyprotein.

The putative 3C-protease and autolytic cleavage sites were identified in this study, which are required for processing polyproteins into functional units, similarly found in other iflaviruses. The virus was maintained in *A. mylitta* populations and upon injection into *A. mylitta* larvae, AmIV spread through systemic infection to silkworm epidermis, midgut, fat body, ovary and eggs. The AmIV infected gravid female moths of *A. mylitta* and *A. proylei* produced AmIV-infected offspring, demonstrating vertical transmission route for AmIV. The study has successfully demonstrated key findings such as discovery of novel member of Iflavirus through genome sequencing molecular characterisation of open reading frames and vertical transmission route for AmIV infecting tasar silkworms, *A. mylitta* and *A. proylei.*

CONCLUDED RESEARCH PROJECTS

AIT 3540: Development of Transgenic Silkworms for the Over- expression of Disease-Resistant Genes for Enhanced Immunity

(collaboration with IISc Bangalore)

G. Ravikumar, Upendra Nongthomba*

Sandhya Rasalkar, Chitra M and Dyna Susan Thomas, Research Fellows

*Indian Institute of Science, Bangalore

Development of silkworm lines with enhanced disease resistance to multiple pathogens to increase silk productivity is the need of the hour. Development of silkworm line resistant to different pathogens through transgenic approach was the objective of the study. The immune genes, Relish 1, Cecropin B, and Drosomycin (from *Drosophila melanogaster*) were used for the investigation. These genes were introduced into piggyBac vector under the independent control of CMV promoter. In addition, three separate constructs with Ceceropin A promoter for Relish1 gene, Cecropin B for Cecropin B gene and Drosomycin promoter for Drosomysin gene were prepared. As a marker, GFP gene was used with ELF promoter with each transgene. The transgene constructs along with helper vector were microinjected or electroporated in to pre-blastoderm eggs of silkworms. The success rate using both the techniques by survival of the transgenic silkworms was 9-16% initially (Table 1). Transgenic silkworms (CSR2 and PM) over-expressing different constructs were reared for 10 generations.

The presence of transgenes and their over-expressions were carried out using GFP screening, PCR, Real Time PCR, Inverse PCR and bioassays (Fig. 13). The GFP expressions were also seen in the cocoons (Fig. 14) apart from egg, larvae and pupae. Presence of all transgenes were detected in all stages of silkworms using amplification by vector specific- and gene specific- primers. The copy number of transgenes was found to be one each. Among various transgenic lines developed, enhanced resistance to pathogens (NPV/*Nosema bombycis*/ bacteria) was observed in the transgenic silkworms carrying Relish followed by Drosomycin. Silkworms carrying Drosomycin were effective against *N. bombycis* causing pebrine disease. The mortality was reduced after infection in the transgenic silkworms (Fig. 16). The survival rate after infection with various pathogens was increased to 50 - 55% in the transgenic lines compared with non-transgenic lines. The economic parameters were improved considerably from first to tenth generation after selection and breeding of transgenic silkworms over generations. Induction of homozygozity and stabilization of generations is underway. Development of the transgenic silkworms pave way to reduce the crop loss due to pathogens and to enhance silk production by using them as parents in the hybrid development programs.

Table1: Comparative effect of transgenesis using microinjection and electroporation techniques in silkworm, *B. mori* strain CSR2

Treatment	No. of Injected /treated Eggs	No. of hatched Eggs	No. of moths G0	GFP Positive larva in G1	% of success
Microinjection	500	150	42	4	9.5
Electroporation	500	10	6	1	16.6

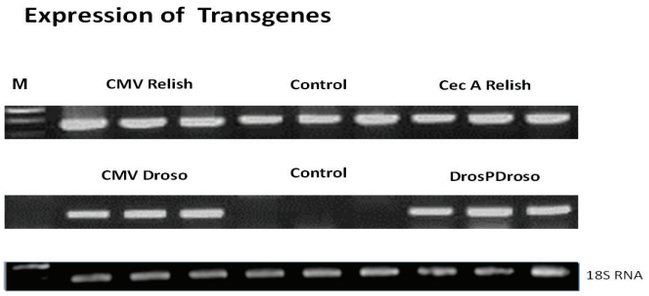


Fig. 13: Expression levels of Relish and Drosomycin genes in transgenic and control silkworm

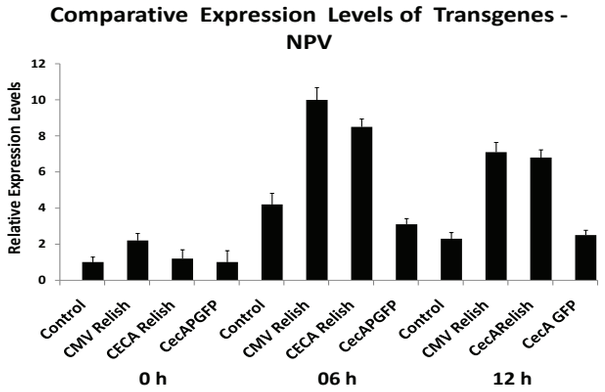


Fig. 14: Over expression of Relish 1 gene in fat body under the control of CMV and CecA promoters after infection in 3rd instar larvae of *B. mori* CSR2 with BmNPV

Fig.15: Expression of GFP transgene in cocoons

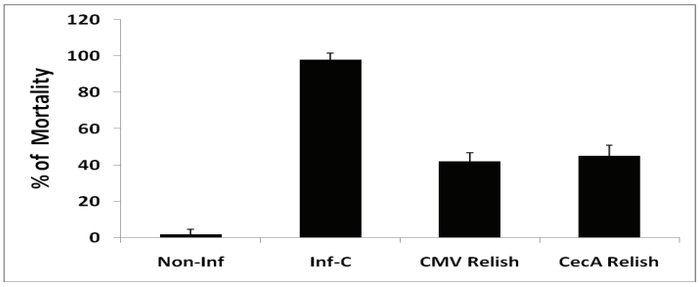


Fig. 16: Mortality rate after infection of 3rd instar larvae of *B. mori* CSR2 with *N. bombycis* showing better tolerance of transgenic silkworms (Non-inf: Non-infected control; Inf-C: infected control; Transgenic CMV Relish and CecA Relish after infection)

Outcome of the project with future action plan

The transgenic silkworms may pave way for enhancing silk production by reducing the crop loss due to pathogens.

AIT 3582: Development of densovirus resistant productive bivoltine silkworm breeds through marker assisted selection

(Collaboration with CSR&TI,Mysore)

Ramesha A, Ponnuvel K.M., Tania Gupta

and **Manthira Moorthy***

*CSR&TI, Mysore

Among different viruses causing Flacherie disease in silkworm, *Bombyx mori* nucleopolyhedrovirus (BmNPV) and *Bombyx mori* densovirus (BmDNV) are the two major prevalent viruses. The *Bombyx mori* Bidensovirus (BmBDV)/ *Bombyx mori* Densonucleosis virus-2 (BmDNV-2) is one of the causative agents of this disease.

A major gene, nsd-2 (non-susceptibility to densovirus-2), a putative BmDNV-2 receptor involved in resistance under recessive mutation condition has previously been identified. The natural deletion occurring in the nsd-2 gene disrupting gene function has contributed to the evolution of BmDNV-2 resistant silkworm breeds. However, nsd-2 has not yet been exploited as a molecular marker or as a transgene for the development of BmDNV-2 resistant breeds. In this study, we have attempted to use the functional/gene marker, nsd-2 for the development of absolute BmDNV-2 resistant productive mulberry silkworm breeds.

A total of 49 productive bivoltine breeder stock/productive breeds were screened and 28 breeds carrying the nsd-2 resistant allele in either heterozygous or homozygous condition were identified. The nsd-2 molecular marker was used for the development of BmDNV-2 resistant productive breeds like CSR6-R, CSR26-R, MASN6-R, MASN7-R and J2P-R.

Among the hybrids generated by crossing DNV-2 resistant parental stocks, CSR6-R CSR26-R and J2P-R showed the complete resistance against the DNV-2 infection suggesting that hybrids generated by crossing homozygous breeds for DNV-2 resistant allele will be completely resistant to DNV-2 infection.

Outcome of the project with future action plan

1. Twenty eight bivoltine Breeders stock/productive germplasms are identified as a carrier of DNV-2 resistant gene, these breeds/germplasm are routinely used in different breeding programme. The outcome of this result can be used to incorporate DNV-2 resistant allele from the identified breeds to develop commercial breeds/hybrids.
2. DNV-2 resistant CSR6-R, CSR26-R, MASN6-R, MASN7-R and J2P-R were developed. Phase-II of the project will be taken up to study in hot spots and also test combination of crosses for new hybrid combination resistant to DNV-2 and also for productive traits.
3. Demonstrated/validated selection for single molecular marker, nsd-2 is sufficient for developing complete DNV-2 resistant silkworm breeds and hybrids without compromising economic traits. Breeders can utilize nsd-2 marker which is easy to perform, efficient and accurate in their breeding programme

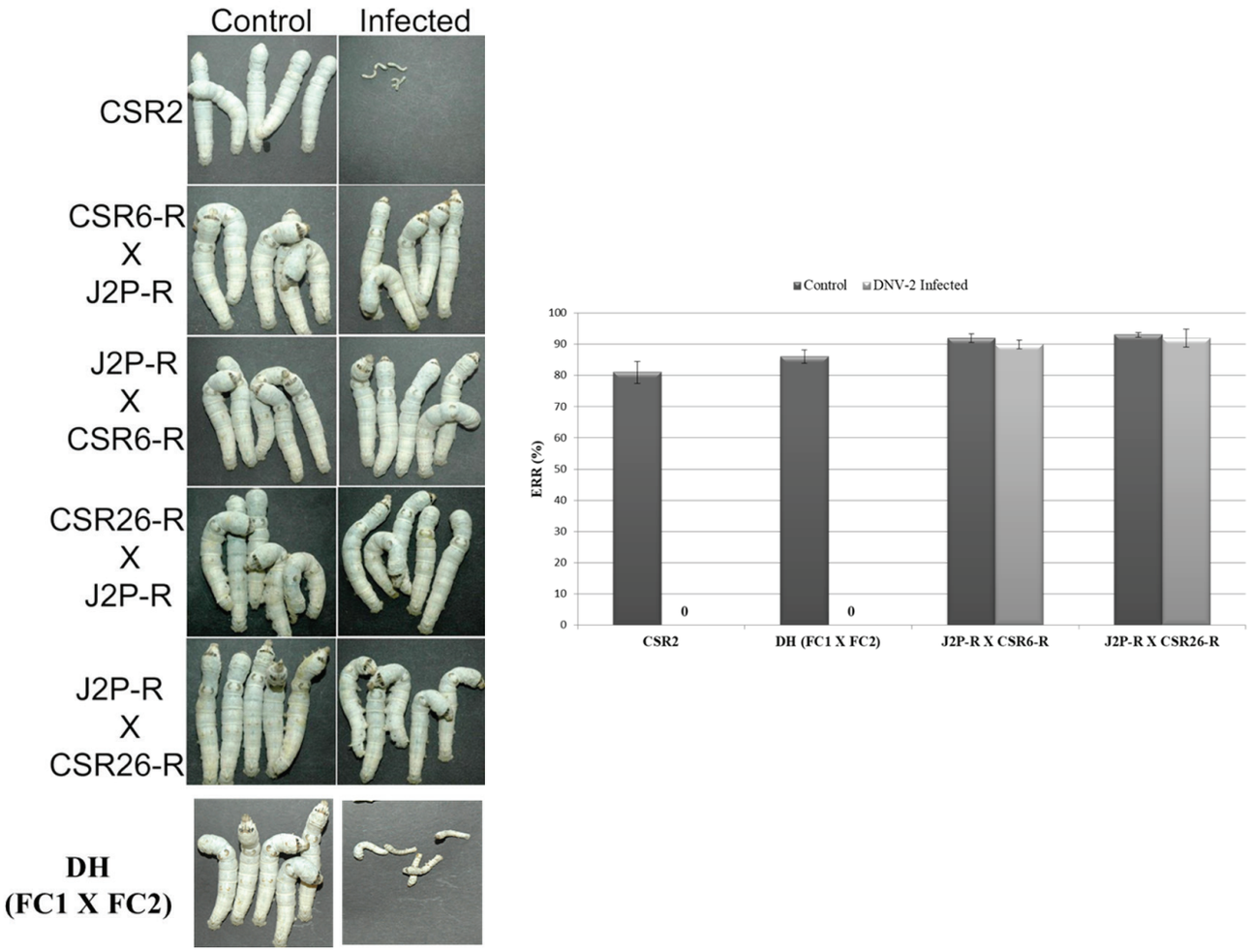


Fig. 17: Validation of BmDNV-2 resistant Hybrids through artificial inoculation.

AIT 5872: Whole Genome Sequencing and functional genomics of Golden Silk Moth *Antheraea assamensis* (Collaboration from CMERTI, Lahdoigarh and technical input from IISc- Bangalore, IIT, Guwahati, CDFD, Hyderabad)

A. R. Pradeep , K.M.Ponnuvel, A. Ramesha , Kartik Neog[*] ,
Upendra Nongthomba[**] , Utpal Bora[#], K.P. Arun Kumar[*] and K.Vijayan[##]

*CMERTI, Jorhat, **IISc, Bangalore, #IIT Guwahati,

##CSB, Bangalore

Muga silkworm (*Antheraea assamensis* Helfer) produces golden silk, which is unique among the varieties of silk. It is lustrous, highly durable, and strongest among all silk fibers with higher tensile strength. However the muga silk production is restricted to North – East (NE) India as its geographical distribution is endemic to Brahmaputra river valley in Assam and Meghalaya. Due to the endemic nature, the muga silkworm underwent strong inbreeding leading to high susceptibility causing large scale disease incidence. On the other hand, the inbreeding seems to have contributed to its silk strength probably due to additive dominance of specific alleles as an adaptive mechanism to protect pupa from the wilderness.

Genetics of muga silkworm has not been analysed in detail. Recently, six populations of

the muga silkworm collected from the West Garo Hills of Meghalaya and commercial crops of Assam were analysed by specific SSR markers which revealed high homozygosity and depletion in genetic variability. The silk proteins and transcriptome variations of this species have not been studied in detail despite the unique properties of golden silk. Moreover no genome sequence information is available for any of the Indian wild silk moths. Hence analysis of the whole genome sequence and functional genomics of the muga silkworm to understand the basic variation from other silkworms, formed the objective of the study. Transcriptome profiling of muga silkworm at different developmental stages or after pathogen infection will be useful to analyse the genetic structure. The information thus generated will be used to investigate association of genes with the quality of muga silk fiber and tolerance to pathogens. Immune genes associated with bacterial infection also could be identified by transcriptome analysis.

(i) Whole genome sequence analysis of the muga silkworm

Single male moths each of the wild type of Muga silkworm, *Antheraea assamensis* collected from Mokokchung, Nagaland and the reared type collected from CMER&TI, Lahdoigarh, Jorhat were used for whole genome sequence analysis. Raw DNA sequence reads from both samples were collected using Illumina and PacBio platforms.

The reads were quality checked using FastQC tool. Data was processed for adapters and low-quality bases. The read statistics showed a total of 156.88 X reads for the cultivar type and 123.51 X reads from wild type from Illumina platform. From the PacBio platform, 4.11X and 4.76 X reads were resolved from cultivar and wild type samples, respectively.

Table 2: Primary alignment statistics to the Bacterial completed DB

Sequence Tag	Total Reads	Processed Read	Mapped to bacterial DB	Percentage of Bacterial Contami-nation	Unmapped to Bacterial DB
SO-6793-Set2-Muga-C1- Ext2-long-insert	87923333	82585545	20218	0.024	82565327
SO-6793-Set2-Muga-C1- Ext2-	85825377	81646036	19354	0.024	81626682
short-insert					
SO-6793-Muga-WT1-Male-Long-Insert	69963188	64801925	50750	0.078	64751175
SO_6793_Muga_ WT1_Male_ Short_ Insert	30085334	28734265	11751	0.041	28722514

In the whole genome data, 0.024 to 0.04% bacterial sequence contaminations were identified and clipped off (Table 2) and then the whole genome sequence is used for secondary assembly. For the final assembly MaSuRCA (Hybrid assembler) was used which showed best assembly (Table 3). The final draft assembly was obtained using Gapcloser program with paired end and mate pair libraries generating a final draft genome. Cultivar sample has genomic size of ~500 Mb with N50 > ~661k and 2456 scaffolds. For the WT1 sample the assembly resulted in genomic size of ~501 Mb with N50 > ~683k and 2697 scaffolds. The secondary assembly is performed using the Platanus assembly program. The assembly generated 1026427 contigs and 138357 scaffolds from wild type whereas 624016 contigs and 116039 scaffolds from cultivar. Final draft assembly using Gap closer program has yielded the following features for the whole genome:

Table 3: Final Draft assembly using Gapcloser Program

	Masurca Contig	Masurca Draft Genome (Scaffold)	Contigs >=1000 bp	Final Draft Genome (Using Gapcloser)
Contigs Generated :	16,067	9,171	2,697	2,697
Maximum Contig Length :	19,12,716	49,53,011	49,53,011	4953275
Minimum Contig Length :	67	101	1,000	1,000
Average Contig Length	31613	54969.5	185746	185827.3
Median Contig Length	455.0	447.0	1,48,028.0	89743
Total Contigs Length	50,79,26,524	50,41,24,852	500957088	501176205
Total Number of Non-ATGC Characters	0	71,04,038	7104038	4185318
Percentage of Non-AT-GC Characters	0.000	1.409	1.418	0.835
Contigs >= 100 bp	16,060	9,171	2,697	2,697
Contigs >= 200 bp	15,962	9,166	2,697	2,697
Contigs >= 500 bp	11,314	5,082	2,697	2,697
Contigs >= 1 Kbp	8,098	2,697	2,697	2,697
Contigs >= 10 Kbp	4,463	1,750	1,750	1,749
Contigs >= 1 Mbp	27	113	113	113
N50 value	2,34,657	6,73,754	6,83,227	683,227

Read usage in assembly: The genomic data generated were aligned back to the assembled genome using bowtie2 and read utilization was observed 95% for both the samples indicating the quality and completeness of the assembly (Table 4). Also on average of 87% RNA-seq reads aligned to the cultivar genome.

Table 4: Read Utilization for the whole genome of cultivar (C1) and wild type 1 (WT1) muga silkworm

Read Utilization	C1			WT1		
	Final Processed Reads	Read Mapped to assembly	% of Read Usability	Final Processed Reads	Read Mapped to assembly	% of Read Usability
SI Reads	81626682	79455103	97.33	28722514	28013601	97.53
LI Reads	82565327	80731483	97.77	64751175	62151807	95.98
MP 5_7	4377259	4324380	98.79	21355830	21206456	99.3
MP_7_10	14233488	14061812	98.78	12988340	12866770	99.06
Pacbio	234308	234308	100	227694	227694	100

(ii) Analysis of transcriptome profile of specific larval tissues of *A. assamensis* after bacterial infection vis a vis control

RNA was extracted from 24 samples comprising control and Aeromonas caviae bacteria-infected samples. RNA of fat body, haemocytes and mid gut were extracted. High purity samples was used for library preparation. Transcriptome analysis of 18 samples from control and infected tissues was performed and sequenced. In addition anterior, middle and posterior silk glands also were analyzed and transcriptome sequencing was performed. The Transcriptome data were mapped into whole genome data and found matching of both sequences confirming the sequences.

From a total of 672.24 million Illumina (150x2) reads, 638.87 high quality reads were used in downstream analysis. Average of 87.14% of the reads aligned to assembled C1 genome. On an average 13054 transcripts were expressed across all samples.

The raw data generated was checked for the quality using FastQC1. Reads were pre-processed to remove the adapter sequences and low quality bases (<q30). Pre-processing of the data is done with Cutadapt2.

Reference Mapping

HISAT23, a splice aligner was used to align the high quality data to the reference genome with the default parameters. All the processed reads were aligned to the assembled cultivar whole genome.

Transcript abundance estimate: On an average 26.61 million reads from 95.15% of high quality data were used for downstream analysis (Table 5).

Table 5: Transcript abundance obtained from different tissues

Sample name	Raw reads	Processed reads	% High quality data
ASG-5D1	27183432	25545468	93.974
ASG-5D1	23448691	23185229	98.876
C1-FB1	28743424	28466125	99.035
C1-FB2	29113809	26938146	92.527
C1-FB3	34659312	32523665	93.838
C1-Hemocytes 1	33055277	30440744	92.09
C1-Hemocytes 2	32801098	30277176	92.30
C1-Hemocytes 3	29229967	28852815	98.70
C1-MG1	26760466	26422339	98.73
C1-MG2	24652440	23867836	96.81
C1-MG3	26309784	24553602	93.32
I1-FB1	30216903	28073510	92.90
I1-FB2	25775567	25454829	98.75
I1-FB3	26831500	25222330	94.00
I1-Hemocytes 1	28376961	25917074	91.33
I1-Hemocytes 2	28726066	25931286	90.27
I1-Hemocytes 3 (repl)	27813870	27644420	99.39
I1-MG1	25812996	24163898	93.61
I1-MG2	23467544	22680227	96.64
I1-MG3	29423224	27449666	93.29
MSG1-5D1	36129694	34400353	95.21
MSG1-5D2	27933738	27504229	98.46
PSG1-5D1	23262509	21586787	92.79
PSG1-5D2	22515497	21776337	96.71

Reference Mapping and expression

All the processed reads were aligned to cultivar assembled genome. An average of 87.14% of the reads was aligned to genome (Fig. 18). On an average 13054 transcripts were expressed.

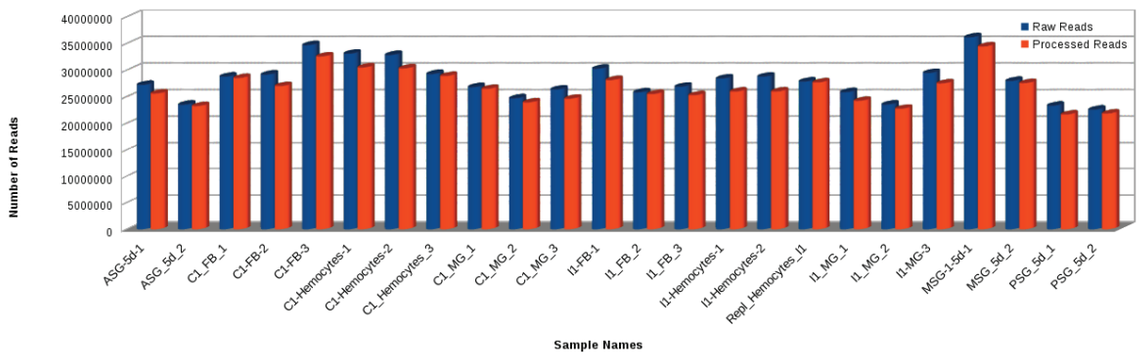


Fig. 18: Read distribution (raw and processed reads) representing the total number of reads generated (in blue color) and high quality reads used for downstream analysis (in red color).

Compiled expression profile at transcript level has been represented in form of Read Count Matrix.

Differential Gene Expression (DGE) analysis: Group wise comparison at transcript level was performed to identify differentially regulated transcripts between control and infected tissues. Sample-wise absolute counts were normalized by DESeq library normalization method. Average expression of samples in a group was considered for fold change calculation. Further analysis on the whole genome and transcriptome data is in progress.

Silkworm Stock maintenance

1. Six lines of Transgenic CSR4 and CSR27 for NPV tolerance
2. Transgenic lines CSR2, CSR4, CSR 27 and PM for fusion protein genes and immune genes
3. Silkworm breeds for NPV/BmBDV resistance developed through marker assisted selection
4. Silkworm races BMI002, BMI003, BMI005, BMI009, BMI034, BMI043, BMI059, BMI063, BMI064 collected from CSGRC, Hosur under the project AIT-3584 for improvement of filament characters.

Trainings Organized

As part of capacity building programme, 21 students belonging to different colleges were given 1-6 months training in the molecular biology techniques, including for M.Sc/M.Tech dissertation work.

Research Advisory Committee (RAC) meeting

25th Research Advisory Committee meeting was held on 27th November 2018 for reviewing the concluded and new project proposals and also to evaluate the progress of the ongoing projects at SBRL under the Chairmanship of Professor P. N. Rangarajan, Department of Biochemistry, Indian Institute of Sciences, Bangalore.

राजभाषा कार्यान्वयन- OFFICAL LANGUAGE IMPLEMENTATION

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

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

(ii) 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 of Hindi notings in the files.

(iii) Joint Hindi workshops was conducted each quarter along with SSTL and RSRS, Kodathi.

(iv) Hindi Pakhwada was celebrated from 14 – 28th September 2018 and various events were conducted.

(v) Two scientists of the Institute (Dr. K.S. Tulsi Naik, Sci-B & Dr. A. Ramesha, Sci-B) passed the Pragya Examination with distinction.

PUBLICATIONS

1. Pawan Shukla[1], Ramesha A. Reddy[1], Kangayam M. Ponnuvel, Gulab Khan Rohela, Aftab A. Shabnam, Shailendra Singh Chauhan, Mrinal K Ghosh and Rakesh Kumar Mishra (2019) Selection of suitable reference genes for quantitative real-time PCR gene expression analysis in Mulberry (*Morus alba L.*) under different abiotic stresses. **Molecular Biology Reports** https://doi.org/10.1007/s11033-019-04631-y.
2. Tania Gupta[1], Ramesha A.[1], Rakesh K. Mishra, Manthira Moorthy, Vankadara Sivaprasad, and Kangayam M. Ponnuvel (2019) Functional marker assisted improvement of productive mulberry silkworm breeds conferring resistance to *Bombyx mori* Bidensovirus (BmBDV). **Agri Gene** 11: 100079
3. Sahar I, Tulsi Naik KS, Rajam MV, Ponnuvel KM and Mishra RK (2019) Targeting immediate early gene (IE1) for inducing virus resistance against grasserie disease caused by BmNPV by RNA interference technology. **Sericologia** 59: 39-44
4. Pawan Shukla[1*], Ramesha A.[1], Kangayam M. Ponnuvel, Gulab Khan Rohela, Aftab Ahmad Shabnam, S. S. Chauhan, Mrinal Kanti Ghosh and Rakesh Kumar Mishra (2018) Comparative analysis of gene expression profiles among contrasting mulberry varieties under cold stress condition. **Journal of Experimental Biology and Agricultural Science.** 6(6): 973-982.
5. Varada B, A.R. Pradeep, A K. Awasthi and K M Ponnuvel (2017) Modulation of NPV gene expression pattern in RNAi- based transgenic silkworm regulate NPV multiplication. **International Journal of Tropical Insect Science (Accepted)**
6. Ravikumar G, DS Thomas, M. Chitra, K. Vijayan and RK Mishra(2018) Development of a sensitive real time PCR assay for the detection of microsporidia in silkworms. **Sericologia** 58(2), 140-143.
7. Esvaran VG, T Gupta, Mohanasundaram A and Ponnuvel KM (2018) Development of isothermal amplification assay for detection of *Nosema bombycis* infection in silkworm *Bombyx mori* targeting polar tube protein 1 gene. **Invertebrate Survival Journal 15**: 352-361
8. Esvaran VG, T Gupta, Nayak ARN, Sivaprasad V and and Ponnuvel KM (2018) Molecular characterization of *Nosema bombycis* methionine aminopeptidase 2 (MetAP2) gene and evaluation of antimicrosporidian activity of Fumagilin-B in silkworm, *Bombyx mori*. **3 Biotech**. 8:386
9. Esvaran VG, Aarthi M, Shruthi M, Tania G and Ponnuvel KM (2018) development and comparison of real time and conventional PCR tools targeting B-tubulin gene for detection of **Nosema** infection in silkworms. **Journal of Parasitic Disease. In Press**

Book Chapter

1. Thammineni Chakradhar, Ramesha A. and Thummala Chandrasekar. Protein Kinases and Phosphatases in Stress Transduction: Role in Crop Improvement: Plant Signaling Molecules (Elsevier)- Accepted

Conference papers

1. Gupta T, Ito K, Kadono-Okuda, Murthy GN, Gowry EV and Ponnuvel KM (2018) Characterization and genome comparison of an Indian isolate of bidensovirus infecting the silkworm *Bombyx mori.* **10th International Workshop on the Molecular Biology and Genetics of the Lepidoptera. Greece,** Aug 19-25, 2018

2. Ponnuvel KM, Ito K, Terenius O, Miranda J and Mishra RK (2018) Molecular characterization of a novel member of Iflavirus infecting *Antheraea mylitta* and *Antheraea proyeli*, the wild silkworm of India. **10th International Workshop on the Molecular Biology and Genetics of the Lepidoptera. Greece,** Aug 19-25, 2018.

3. Shambhavi P Hungund, Pooja Makwana, Appukuttan Nair R Pradeep and Rakesh K. Mishra (2019). Microsporidian infection in *Bombyx mori*: Molecular events and new targets to control infection. Research paper presented at **Asia-Pacific Congress of Sericulture and Insect Biotechnology (APSERI-2019);** 2- 4 March 2019

4. Ravikumar G and Mishra RK (2019) Vitellogenin receptors in silkworms:Molecular analysis and their role in egg production. Research paper presented at **APSERI-2019;** 2- 4 March 2019

5. Chithra M, Thomas D, Rasalkar S, Vijayan K, Mishra RK and Ravikumar G (2019) Recombinant expression of silk fibroin fusion protein in Pichia pastoris. Research paper presented at **APSERI-2019**; 2- 4 March 2019

6. Thomas D, Rasalkar S, Chithra M, Upendra N Mishra RK and Ravikumar G (2019) Development of transgenic silkworm *Bombyx mori* expressing Drosomycin. Research paper presented at **APSERI-2019**; 2- 4 March 2019

7. Ramesha A.[1], Tania Gupta[1], Kangayam M. Ponnuvel, Appukuttannair Pradeep, Rakesh K. Mishra, Manthira Moorthy, Vankadara Sivaprasad (2019) Loss of function in susceptibility gene: utilization in development of *Bombyx mori* Bidensovirus (BmBDV) resistant productive silkworm breeds. Research paper presented at **APSERI-2019**. 1Co-First authors

8. Vijayan K, Ramesha A. and Mishra R.K. Contribution of breeding and biotechnology to crop improvement in mulberry (*Morus* spp.). Lead paper presented at **APSERI-2019.**

9. K. S. Tulsi Naik, Sahar Ismail, K M Ponnuvel and R,K Mishra (2019) A stable and efficient method to produce dsRNA to elicit RNA interference against BmNPV infection in silkworm *Bombyx mori-L* A Transkingdom RNAi approach **APSERI-2019.**

10. K. S. Tulsi Naik, Shambhavi Hungund M.V. Rajam, K M Ponnuvel, R K Mishra (2018) A Bacterial Transkingdom RNAI Approach for expression of dsRNA in Silkworm *Bombyx mori.L* to elicit RNA interference against Viral genes associated with BmNPV infection, **IISF, Young Scientist conference Lucknow**

ii Workshop / Trainings attended

1. **Dr K. S. Tulsi Naik and Dr A. Ramesha** - Workshop on Communication and presentation skills organized by CSB at Centre of excellence from 29th -30th May 2018
2. **Dr K. M. Ponnuvel, Dr G. Ravikumar, Dr A. R. Pradeep, Dr K. S. Tulsi Naik and - Dr A. Ramesha** attended Orientation Training Programme for Seed Officers and Seed Analysts organized by NSSO, Bangalore during 17th to 22nd December 2018 at SSTL, Bangalore
3. **Dr A. R. Pradeep** attended National Training Programme on "The issues and policies related to GMOs/GM Crops in India" at UAS , GKVK Bangalore during 4-8th February 2019

RESEARCH ADVISORY COMMITTEE

Sl No	RAC Members	Remarks
1	Prof. P.N. Rangarajan Dept. of Biochemistry, Indian Institute of Science Bangalore	**Chairperson**
2	Prof. Upendra Nongthomba Dept. of Molecular Reproduction and Developmental genetics Indian Institute of Science Bangalore	**Member**
3	Dr. Malali Gowda Professor Transdisciplinary University Yelahanka, Bangalore	**Member**
4	Prof. Anitha Peter Professor, Dept. of Plant Biotechnology, UAS, GKVK, Bangalore	**Member**
5	Dr. R. K. Mishra Seri-Biotech Research Laboratory Kodathi, Carmelram post, Sarjapur Road Bangalore	**Member- Convener**

Institute Bio safety Committee (IBSC)

Sl no	Name and Address	Remarks
1	**Prof. Usha Vijayaraghavan** Department of Microbiology & Cell Biology, Indian Institute of Science, Bangalore	Member-Secretary
2	**The Director** Seri Biotech Research Laboratory, Bangalore	Chairperson
3	**Dr. R. Ashokan** Principal Scientist Dept. of Biotechnology IIHR, Hesaraghatta Bangalore	Member
4	**Dr.B. L.Mohan Kumar,** MBBS AMA-CSB Employees Dr Mohan Nursing Home,MBBS Sarjapura Road, Bangalore	Member
5	**Prof. H.P. Puttaraju** Department of Life Sciences, Bangalore University	Member
6	**Dr. V.V. Satyavati** Molecular Genetics Lab CDFD, Hyderabad	Member -External Expert
7	**Dr. A. R. Pradeep** Scientist D, Seri biotech Research Laboratory, Bangalore	Member -Internal Expert

HUMAN RESOURCES

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

RESEARCH FELLOWS/ASSISTANTS

Sl No	Name	Designation
1.	Mr.Wazid Hussain	Research Associate (CSIR)
2	Mrs Varada Burdekar	Senior Research Fellow
3	Ms Tania Gupta	Junior Research Fellow
4	Mrs Vijaya Gowri	Junior Research Fellow
5	Mrs Shambhavi P Hungund	Junior Research Fellow
6	Mrs Dyna Susan Thomas	Junior Research Fellow
7	Mrs Chitra Manoharan	Junior Research Fellow
8	Mrs.Sandhya Rasalkar	Junior Research Fellow
9	Ms Shruti M	Junior Research Fellow
10	Ms. Sahar Ismail	Junior Research Fellow
11	Ms Gopika Padmakumar	Junior Research Fellow
12	Ms Aarti	Project Assistant
13	Ms Diksha khajje	Project assistant
14	Mr Naresh Kumar	Rearing Assistant

ADMINISTRATION

Sl No	Name	Designation
1	Mrs. Shyamala Murthy	Asst. Director
2	Mr. Mohan Raj	Asst. Superintendent (up to 02.06.2018
3	Mr Chandrashekhar Rao	Asst. Superintendent
4	Mr A. Mallesha	Asst. Technician
5	Mr. S. Nagesh	Driver
6	Mr.Kenchappa	Multi tasking Staff

Superannuation


Shri. S. N. Gundu Rao, Technical Assistant and Shri Mallesha, Asst. Technician retired from service of Central Silk Board (Government of India) on attaining superannuation at the age of 60 years. The fraternity of SBRL thank them for their esteemed service and wish them happy retired life.

Financial Progress (2018-19)

During the year under report, an expenditure of Rs. 330.15 lakhs, under following heads, was incurred from Grants-in-Aid sanctioned by CSB and from funds received from Department of Biotechnology, Government of India.

S No.	Particulars	Amount (Rs. In lakhs)
1	Salary and allowances	187.08
2	Wages and EPF	17.06
3	Travelling Expenses	2.18
4	Contingent expenses	31.09
5	Assets	9.73
6	Others	56.31
	Subtotal	**303.99**
7	DBT assistance	26.16
	Total	**330.15**

Other Events

Vigilance Awareness week

Vigilance Awareness week was organized from 31st October to 3rd November 2018 as per instruction of Government of India and Central Silk Board. A debate competition on the theme "Eradicate Corruption-Build a New India" was also conducted and the winners were awarded.

Swachhta Pakhwada

Swachhta pakhwada was organised from 01.03.2019 to 15.03.2019 jointly with Silkworm Seed Technology Laboratory, (SSTL), Kodathi and various programmes were organised as per Central Office instructions.