वार्षिक प्रतिवेदना
ANNUAL REPORT
2017 - 2018

रेशमकीट जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला
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Annual Report 2017-18

March 2019

English	:	100 copies
Published by	:	**Dr. R. K. Mishra** Director Seribiotech Research Laboratory Bengaluru - 560 035.
Editorial Committee	:	**Dr. K. S. Tulsi Naik** **Dr. A. Ramesha** **Dr. A. R. Pradeep** **Dr. K. M. Ponnuvel**
Hindi Translation	:	**Dr. K. S. Tulsi Naik** **Mrs. Meena Kamath** (C.O., CSB)
Cover Pages	:	Eggs and pupa of transgenic silkworm *Bombyx mori* expressed Green fluorescence protein with cec A promoter developed at SBRL through germline transformation by microinjection
Printed at	:	**Creative Graphics** # 149, I Floor, "Somanna Complex" Sultanpet, Bengaluru - 560 053. Ph : 080 - 2237 0262

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प्रक्कथन

मुझे रेशम जैव प्रौद्योगिक अनुसंधान प्रयोगशाला की वार्षिक रिपोर्ट के वर्तमान खण्ड को प्रस्तुत करने में असीम प्रसन्नता है, जो इस प्रयोगशाला द्वारा वर्ष 2017-19 के दौरान किए गए योगदान और अनुसंधान के बहुरुपदर्शक दृश्य प्रस्तुत करता है। यह उल्लेखनीय है कि प्रयोगशाला द्वारा किए गए रेशम जैव प्रौद्योगिकी आविष्कार वर्तमान में धीरे-धीरे फलदायी/स्थानांतरीय परिणामों में परिवर्तित हो रहे हैं, जिससे रेशम की पैदावार में सुधार हुआ है । अंतरराष्ट्रीय और राष्ट्रीय साझेदारी के माध्यस से सुदृढ़ किए गए अत्याधुनिक अनुसंधान के अतावा, जैव प्रौद्योगिकी में प्रतिभा का विकास प्रमुख गतिविधियों मे से एक है ।

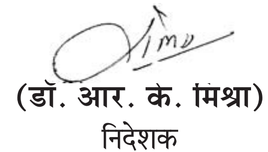
वर्ष के दौरान कुछ महत्वपूर्ण अनुशंधान व विकास योगदानों में न्यूक्लियोपॉहाईड्रोवायरस (बीएमएनपीवी) से प्रतिरक्षा के लिए आणविक मार्कर सहायता प्रास चयन द्वारा विकसित एमएएसएन नस्लों का उपयोग करते हुए तैयार किए गए संकरों को क्षेत्र परीक्षण शामिल हैं । आरंभिक रिपोर्ट, दक्षिणी और पूर्वी भारत के रेशम उत्पादन क्षेत्रों में एमएएसएन संकरों की सफलता को दर्शाती हैं । नस्ल सुधार के प्रति इसी प्रकार के प्रयोगों में फ्लचेरी - कारक डीएनवी2 विषाणु के विरूद्ध सहिष्णु सीएसआर6 और सीएसआर26 वंशों के विकसित करना एवं रोगजनक के संक्रमण से रेशमकीट को प्रारंभिक सुरक्षा प्रदान करने लिए ट्रांसजेनिसिस के माध्यम से प्रतिरक्षित प्रोटीन की अभिव्यक्ति शामिल है । इस दिशा में ट्रांसकिंगडम आरएनए हस्तक्षेप के माध्यम से एनपीवी संक्रमण का दमन एक और अभिगम है ।

वन्य क्षेत्र के लिए, ओक तसर रेशमकीट के टाइगर बैंड रोग के वायरस के पूरे जीनोम अनुक्रस विश्र्लषण से इसके समूह - 1 अल्फा-बैकुलोवायरस, एन्थीरिया प्रायली न्यूक्लियोपॉलीहाइड्रो विषाणु के एक सदस्य के रूप जानकारी मिली। इसी प्रकार, भारतीय मूगा रेशमकीट एन्थीरिया असमेन्सिस के पूरे जीनोम अनुक्रम विश्र्लषण से जीनोम का आकार - 500 एमबी दर्शाया और लगभग 24000 जीन ट्रांसक्रिप्शनल रूप से सक्रिय लगे । भारत में भूगा रेशमकीट को बेहतर बनाने के लए उक्त बुनियादी जारकारी का उपयोग हमारे समक्ष जैव-प्रौद्योगिकीय जुनौती होने जा रहा है ।

फाईब्रोइन - सेक्रोपिन संलयन अनुक्रम और इसके प्रोटीन अभिव्यक्ति युक्त ट्रांसजेनिक रेशमकीट का सफल संश्र्लष रेशम का नए जैव चिकित्सा अनुप्रयोगों का संकेत है । इस दिशा में अन्य कार्य प्रगति पर है ।

प्रशीक्षित मानव संसाधन की दिशा में, 10 अध्येताओं और 18 छोत्रों को जैव प्रौद्योगिकी विधियों और अनुप्रयोगों में प्रशिक्षण प्रदान किया गया है ।

यदि मैं अनुसंधान एवं विकास प्रयासों में वैज्ञानिकों, अध्येताओं, तकनीकी सहायकों और प्रशासनिक अनुभाग की टीस द्वारा किए गए उल्लेकनीय प्रयासों की सराहना न करुँ तो मैं अपने कर्तव्य में विफल रहूँगा । सदस्य सचिव, केन्द्रीय बोर्ड ओर जैव प्रौद्योगीकी विभाग, वैज्ञानिक और औद्योगिक अनुसंधान परिषद और विज्ञान और प्रौद्योगिकी मंत्रालय (भारत सरकार) से निरंतर वित्तीय सहायता और आईआईएससी, बेंगलूर जैसी विभिन्न राष्ट्रीय प्रयोगशालाओं से अनुसंधान समर्थन ने हमारी शोध गतिविधियों को अधिक क्षमता प्रदान की है और हम उनके निरंतर समर्थन के लिए आभारी हैं ।



(डॉ. आर. के. मिश्रा)
निदेशक

FOREWORD

It gives me immense pleasure in presenting the present volume of the Annual Report of SBRL, which gives a kaleidoscopic view of the contributions made and research undertaken by it during the year 2017-18. It is worth mentioning that the seri-biotechnological inventions made by the lab over the period are presently converting gradually into fruitful /translational results, leading to improved silk yield. Besides cutting edge research strengthened by international and national partnerships, development of talent pool in Biotech is one of the major activities.

Some of the important R&D contributions during the year include taking up field trials of the hybrids prepared using the MASN breeds developed by molecular marker assisted selection for tolerance against the nucleopolyhedrovirus (BmNPV). Initial reports point to success of the MASN breeds in sericultural belts of Southern and eastern India. Similar approaches towards breed improvement include developing tolerant CSR6 and CSR26 lines against flacherie-causing DNV2 virus, over expression of immune proteins through transgenesis to provide early protection of silkworm from pathogen attack. Suppression of NPV infection through Trans-kingdom RNA interference is yet another approach in this line.

For the Vanya sector, whole genome sequence analysis of the Tiger Band disease causing virus of Oak tasar silkworm revealed it as a member of group I alpha-baculovirus, *Antheraea proylei* nucleopolyhedrovirus. Similarly, whole genome sequence analysis of the Indian Muga silkworm, *Antheraea assamensis* showed ~500mb as the genome size and nearly 24000 genes seem transcriptionally active. Utilization of the basic information to improve the muga silkworm in India is going to be one of the biotechnological challenges before us.

Successful synthesis of transgenic silkworm carrying construct with fibroin – cecropin fusion sequence and its protein expression explore new biomedical applications of silk. Further works in this line are under way.

Under trained human resource development front, 10 research fellows and 18 students have been provided training in biotechnology tools and applications.

I would fail in my duty, if I do not appreciate the remarkable efforts made by the team of Scientists, research fellows, technical assistants and administrative wing in the R&D endeavors. Constant support of the Member Secretary, CSB and Financial support from Department of Biotechnology, Council of Scientific & Industrial Research and Ministry of Science & Technology (Government of India) and the research support from different national laboratories like IISC, Bengaluru has made our research activities gain more potential and we owe them for their constant support.

(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 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 परिणाम की मुख्य विशेषताएं

1. सरुजत और सीएसआर2 के पैतृक के रुप में तथा सीएसआर2 को आवर्ती पैतृक के रूप में उपयोग करते हुए बैकक्रॉस प्रजनन के माध्यम से एनजीवी सहिष्णुता के लिए मार्कर सहायता प्राप्त चयन वंश पूर्व में ही विकसित किए गए । नये संकर, बेहतर एरपीवी प्रतिरोध के साथ-साथ उच्च उपज लक्षण दर्शाये और इस तरह एनपीवी सहिष्णुता तथा उपज विशेषक में संतुलित लक्षण दर्शाये । ये एमएएसएन नस्ल तीन जीन नामत: डीऑक्सी काइनेज (नाग 88), प्रोटीन काइनेज जी-1 बी (नाग 65) और ग्लूकोसाइलट्रांसफेरेज़ (नाग 84) से उत्पन्न विशिष्ट मार्करों की उपस्थिति से लक्षित होती है । एमएएसएन वंश के अंडों (रोमुच) की आपूर्ति केरेअवप्रसं, मैसूर, बरहमपुर और पाम्पोर को की गई । इन वंशों में किया गया और देश के विभिन्न रेशम उत्पादन क्षेत्रों में नए एमएएसएन वंश के अनुकूलह क्षमता को दर्शाने के लिए बेहतर वंश में मार्करों की उपस्थिति की पुष्टि की जाती है ।

2. वन्य और कीटपालित भारतीय मूगा रेशमकीट, एन्थीरिया असमेंसिस के पूरे जीनोम को इलुमिना और पैकबॉयो प्लेटपॉर्म पर अनुक्रमित किया गया है और जीनोम का आकार 2456 स्केफोल्ड में - 500 एमबी में फैला पाया गया है, जो अन्य लेपिडोप्टेरान की तुलना में बड़ा है । अंतिम मसौदा जीनोम को निम्न गुणवत्ता का रीड हटाकर तथा अंतराल कम करने के कार्यक्रम का उपयोग करते हुए तैयार किया गया । वन्य में, कुल कॉंटिग लंबाई 501176205 थी जिसमें अधिकतम लंबाई 4953275 और औसत लंबाई 185827.3 थी। कल्टीवर प्रकार में, कुल कॉंटिग लंबाई 500314777 थी जिसकी अधिकतम लंबाई 3198103 और औसत लंबाई 203711.2 थी ।

3. पुन: संयोजक फाईब्रॉइन -सेक्रोपिन फ्यूज्ड सीक्वेंस बनाया गया और फ्यूजन प्रोटीन को पाइकियापास्टोरिस सिस्टम में व्यक्त किया गया । फ्यूजन प्रोटीन उत्पादन को बढ़ाने की प्रक्रिया प्रगति पर है ।

4. प्रतिरक्षा जीन की अधिक अभिव्यक्ति के लिए निर्मण के साथ ट्रांसजेनिक रेशमकीट के तीन वंश, एक रिपोर्टर जीन के साथ ग्रीन फ्लोरेसेन्ट प्रोटीन (जीएफपी) के साथ रिलिश1, ड्रोसोमाइसिन और सेक्रोपिन बी को शुद्ध मैसूर और सीएसआर2 से रोधक्षमता के लिए संश्लेषित किया गया । अंडों में निर्मण के सूक्ष्म इंजेक्शन द्वारा जर्मलाइन परिवर्तन के माध्यम से ट्रांसजेनिक वंशों का संश्लेषण किया गया । चयन और इनब्रीडिंग द्वारा ट्रांसजेनिक रेशमकीटों का प्रगुणन प्रगति पर है ।

5. ओक तसर रेशमकीट एन्थीरयिा प्रॉयली एक अज्ञात विषाणु के कारण होने वाले टाइगर बैंड रोग से प्रभावित होता है । विषाणु को शुद्ध किया गया और जीनोम को अनुक्रमित किया गया । विषाणु जीनोम के पूर्ण अनुक्रमण (अभिगम: जीआई: 1371952746) के माध्यम से, कारक सूक्ष्मजीव की पहचान एन्थीरिया प्रॉयली न्यूक्लियो पॉलीहिडरो विषाणु (एएनआरएनपीवी) के रूप में की गई जो कि समूह 1 आल्फाबेकलॉ विषाणु के हैं । जीनोम के छह संरक्षित क्षेत्रों ने एन्थीरियापर्णी एनपीवी (एएनपीएनपीवी) के साथ होमोलॉजी

साझा की गई और उनमें से एक, पी94 जीन सेगमेंट ने नीरंतर प्रवर्धन दिखाया ओर मुख्य रुप से एएनपीआरएनपीवी संक्रमण के शीघ्र पता लगाने के लिए चुना गया ।

6. एनपीवी प्रगुणह पर एनपीवी जीन के विरुद्ध डीएसआरएनए के प्रभाव को वैद्य करने के लिए, एनपीवी-संक्रमित जिम्भक को खिलाते हुए जैव आमापन किया गया, जिसमें एनपीवी संक्रमण के प्रति प्रतिरोध और एनपीवी संक्रमित डिम्भक में <5% उत्तरजीविता की तुलना में 30-40% उत्तरजीविता देखी गयी । एनपीवी प्रगुणह का परीक्षण एनपीवी प्रगुणन जीन लेफ1, आईइ1, लेफ3, जीपी64 और जीपी41 की अभिव्यक्ति प्रोफाइल की जांच कर एनपीवी का प्रगुणन किया गया । डीएसआरएनए पोषित डिम्भक में एनपीवी जीन की अभिव्यक्ति एनपीवी संक्रमित नियंत्रक जिम्भक की तुलना में कम दर पर था, जो विषाणु जीन अनुलेखन स्तर तथा कम विषाणु प्रगुणन पर जीएसआरएनए के निरोधात्मक प्रभाव को दर्शाया ।

7. विभिन्न बहुप्रज अभिगमों में तंतु लक्षणों के साथ जीन / मार्कर की संगति को पहचानने और विश्लेषण करने के लिए, विशिष्ट इंट्रोन और एक्सॉन वेम्प्लिकॉन के डीएनए अनुक्रमण का निष्पादन किया गया । तंतु लंबाई (जीएफएल 1) के लिए जीन के विश्लेषण ने बहुप्रज प्रजातियों में 600 बीपी एम्प्लिकॉन ओर द्विप्रज प्रजाति सीएसआर2 में 700 बीपी एम्प्लिकॉन दिखाया । सभी बहुप्रज प्रजातियों में द्विप्रज प्रजातियों की तुलना में लगभग 100 बीपी का विलोपन देखा गया । इस क्रम के एनसीबीआई-बीएलएएसटी विश्लेषण से बॉम्बिक्स मंडारिना के फाइब्रॉइन-एच इंट्रोन क्षेत्र के साथ 81% समानता देखी गयी । बहुप्रज और द्वविप्रज प्रजातियों के बीच एक फ्रॉस के एफ1 को दोनों युग्मविकल्पी विरासत में मिलीं ओर एफ2 में अलग की गई।

8. बीएमबीडीवी (डीएनवी2) की पहचान रेशमकीट में फ्लैचरी रोग पैदा वाले प्रमुख रोगजनकों में से एक के रूप में की जाती है । 49 वाणिज्यिक रुप से महत्वपूर्ण प्रजातियों के जीनप्ररुपण के माध्यम से, डीएनवी2 प्रतिरोधी युग्मविकल्पी (एनएसडी2) धारक 28 उत्पादक नस्लों की पहचान की गई । नर और मादा पैतृकों का जीनप्ररुपण किया गया और डीएनवी2 प्रतिरोधी सीएसआर6 और सीएसआर26 धारक एनएसडी-2 प्रतिरोधी युग्मविक्लजी समयुग्मजी दशा में विकसित किए गए । ये सीएसआर 6 आर और सीएसआर 26 आर वंश डीएनवी2 संक्रमण के लिए प्रतिरोधी और उच्च उपन देने वाले लक्षणों के साथ है जिसका उपयोग डीएनवी2 प्रतिरोधी प्रजनन कार्यक्रमों के लिए पैतृकों के रुप में किया जा सकता है ।

9. एनपीवी सहिष्णु एमएएसएन वंश, एमएएसएन-6, और एमएएसएन-7 में एनएसडी-2 प्रतिरोधी युग्मविकल्पी के लिए समयुग्मजी दशा में नर और मादा पैतृकों का स्क्रीनिंग की गयी । डीएनवी2 के साथ संरोपण के बाद, एमएएसएन-6 और एमएएसएन-7 ने 70-80% प्रभावी कीटपालन दर दिखाया, जिससे बेहतर उपज लक्षणों के साथ बहुविषाणु (एनपीवी और जीएनवी2) सहिष्णु प्रजातियां विकसित की गईं ।

RESEARCH HIGHLIGHTS

1. Marker assisted selection lines for NPV resistance (MASN) were developed earlier by backcross breeding using Sarupat and CSR2 as parents and with CSR2 as recurring parent. The new breeds showed better NPV tolerance and high yield traits and thereby showed balanced traits at NPV resistance as well as yield traits. These MASN breeds are characterized by presence of specific markers derived from three genes namely *deoxy kinase* (Nag 88), *protein kinase G-1B* (Nag65) and *glucosyl transferase* (Nag 84). The MASN line eggs (DFLs) were supplied to CSRTI-Mysore, Berhampore and Pampore. The lines were successfully reared at different regions including Coonoor, Jammu, Dehradun, Berhampore and Bangalore and selected MASN4 as better line in terms of survival showing the adaptability of the new MASN line at different sericultural regions of the country. Presence of the markers is confirmed in the MASN lines provided to different regions.

2. Whole genome of the wild and reared Indian muga silkworm, *Antheraea assamensis* is sequenced on Illumina and PacBio platforms and found the genome size as ~500 mb spread in 2456 scaffolds which is bigger than other lepidopterans sequenced.The final draft genome was prepared by removing the low quality reads and using gap closer program. In wild type, total contig length was 501176205 with maximum length of 4953275 and average length of 185827.3. In cultivar type, total contig length was 500314777 with maximum length of 3198103 and average length of 203711.2.

3. Construct of recombinant fibroin – Cecropin fused sequence was made and the fusion protein was expressed in *Pichia pastoris* system. The process of scaling up of fusion protein production is in progress.

4. Three lines of transgenic silkworms with construct for over expression of immune genes, *relish1, drosomycin* and *cecropin B* along with a reporter gene encoding green fluorescent protein (GFP) were synthesized from Pure Mysore and CSR2, for enhanced immunity. Transgenic lines were synthesized through germline transformation by microinjection of the construct in the eggs. Multiplication of the transgenic silkworms by selection and inbreeding is in progress.

5. Oak tasar silkworm *Antheraea proyeli* is affected by tigerband disease caused by an unknown virus. The virus was purified and the genome was sequenced. Through complete sequence (Accession: GI: 1371952746) of the viral genome, the causative microbe was identified as *Antheraea proylei* nucleopolyhedrovirus (AnprNPV) belong to group I alphabaculovirus. Six conserved regions of the genome shared homology with *Antheraea pernyi* NPV (AnpeNPV) and one of them, the p94 gene segment showed consistent

amplification and primarily selected as candidate for early detection of the AnprNPV infection.

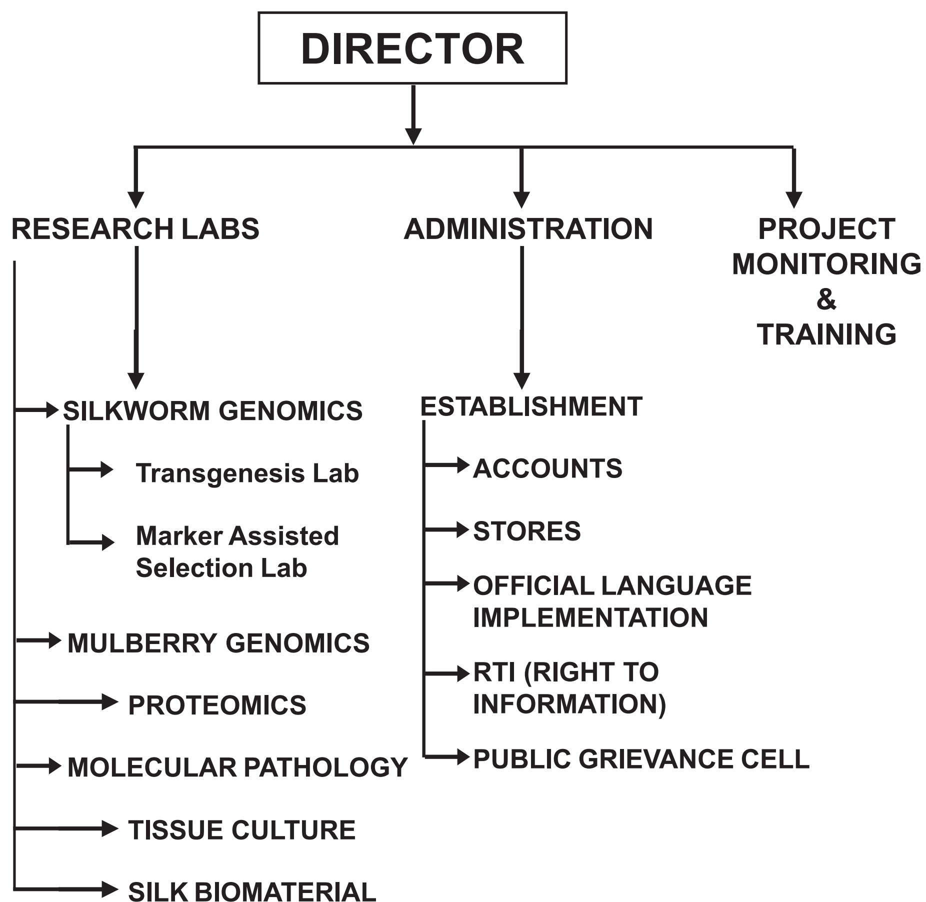
6. In order to validate the effect of dsRNA against NPV genes on NPV multiplication, bioassay was performed by feeding NPV- infected larvae with the dsRNA which showed resistance to NPV infection and 30- 40% survivability of infected larva in comparison with <5 % survival in NPV- infected larvae. NPV multiplication was tested by examining the expression profile of NPV multiplication genes *lef1*, *ie1*, *lef3*, *Gp64* and *Gp41.* In dsRNA fed larvae, NPV gene expression was at a lower rate compared to NPV- infected control larva indicating inhibitory effect of dsRNA on viral gene transcript level and decreased viral multiplication.

7. In order to identify and analyze association of candidate genes / markers with the filament characters in the divergent multivoltine accessions, DNA sequencing of amplicons of specific introns and exons was performed. Analysis of *gene for filament length (GFL 1*) showed 600bp amplicon in multivoltine races and 700bp amplicon in the bivoltine race CSR2. A deletion of approximately 100bp is observed in all the multivoltine races in comparison with bivoltine races. The NCBI- BLAST analysis of this sequence showed 81% similarity with the Fibroin –H intron region of *Bombyx mandarina.* F1 individuals of a cross between multivoltine and bivoltine races inherited both the alleles and segregated in F2 population.

8. *Bm*BDV (DNV2) is identified as one of the major pathogens causing flacherie disease in silkworm. Through genotyping of 49 commercially important races, 28 productive breeds carrying DNV2 resistant allele (*nsd2)* were identified. Male and female parents were genotyped and developed DNV2 resistant CSR6 and CSR26 carrying *nsd-2* resistant allele in homozygous condition. These CSR6R and CSR26R lines are resistant to DNV2 infection and with high yielding traits which can be used as parents for DNV2 resistant breeding programs.

9. Screening of male and female parents for *nsd-2* resistant allele in NPV tolerant MASN lines, MASN-6 and MASN-7 carry *nsd-2* resistant allele in homozygous condition were developed. After inoculation with DNV2, MASN-6 and MASN-7 showed 70-80% effective rearing rate thereby developed multi virus (NPV and DNV2) tolerant races with better yield traits.

ORGANIZATION CHART



LIST OF RESEARCH PROJECTS

ON-GOING RESEARCH PROJECTS

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

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

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

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

5. **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)** (Sep. 2016 - Aug. 2019)

6. **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. (Network project of SBRL, CSRTI - Mysore, CSRTI - Berhampore and CSRTI - Pampore, funded by Department of Biotechnology, Govt of India, New Delhi) (Feb. 2017 - Jan. 2020)

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

8. **AIT 5872:** Whole Genome Sequencing and functional genomics of Golden Silk Moth *Antheraea assamensis* **(In Collaboration with IISc, Bangalore, IIT, Guwahati, CDFD, Hyderabad, CMERTI, Ladoigarh)** (Oct. 2015 – Sep. 2018)

CONCLUDED RESEARCH PROJECT

1, **ARP 3518**: Expression profiling of genes associated with resistance to *Beauvaria bassiana* in *Bombyx mori* silkworm strains **(In collaboration with CSGRC, Hosur)** (Aug. 2014 - Sep. 2017)

PROGRESS OF ON-GOING RESEARCH PROJECTS

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

Duration: **May 2015 – April 2019**

G. Ravikumar and K. Vijayan [CSB, Bangalore]
Mrs Chitra, M. and Mrs. Dyna Susan Thomas

Aim: To develop novel fibroin fusion protein for new generation dressing materials and tissue engineering applications.

The recombinant fibroin fusion protein was expressed in *Pichia pastoris*. In order to get more fusion protein for various assays, the Pichia culture was scaled up with altering culture conditions. The detection and purification of recombinant proteins from fresh Pichia cultures was completed by denaturing IMAC. Proteins were subjected to refolding. The refolded proteins are being subjected to SDS-PAGE and western blotting. A mammalian cell culture facility is being set up for various assays using the recombinant fusion protein.

AIT 3540 : Development of Transgenic Silkworms for the Over - expression of Disease-Resistant Genes for Enhanced Immunity (In collaboration with IISc Bangalore)

Duration: **July 2015 – June 2018**

G. Ravikumar and Upendra Nongthomba [IISc, Bangalore]
Mrs. Sandhya Rasalkar, Mrs. Chitra M, Mrs. Dyna Susan Thomas [JRFs]

Aim: Development of silkworm lines with enhanced disease resistance to multiple pathogens to increase silk productivity.

Transgenic silkworms (CSR2 and PM) over-expressing CMV Relish, CecA Relish were screened and positive silkworms were cross mated. G3 generation is underway. Silkworms possessing *Drosomycin* and*Cecropin B* transgenes were found week and many of them died. Hence, repeated the microinjection procedure for fresh stock. Observations are under progress. Facility for the development of transgenic silkworm was set up at SBRL.

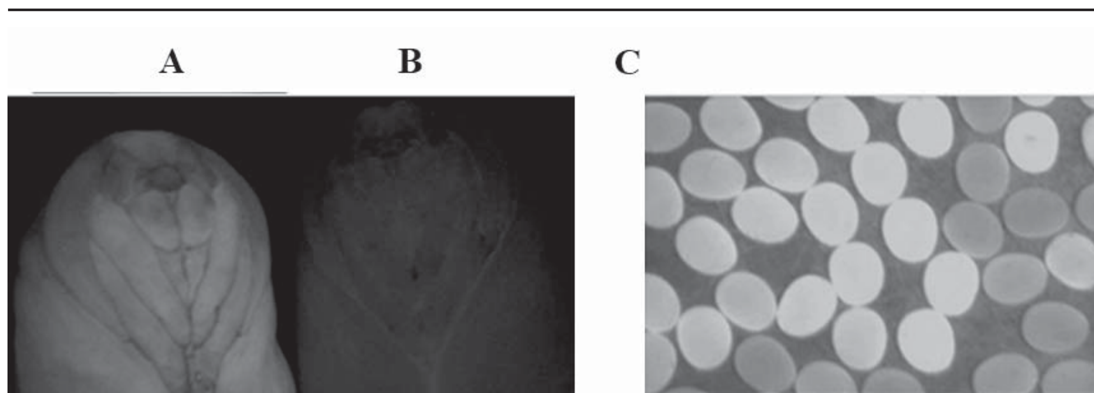


Figure - 1: (CSR2) Transgenic silkworm expression of GFP in pupa (A), Non-transgenic control pupa (B) and transgenic silkworm eggs (C)

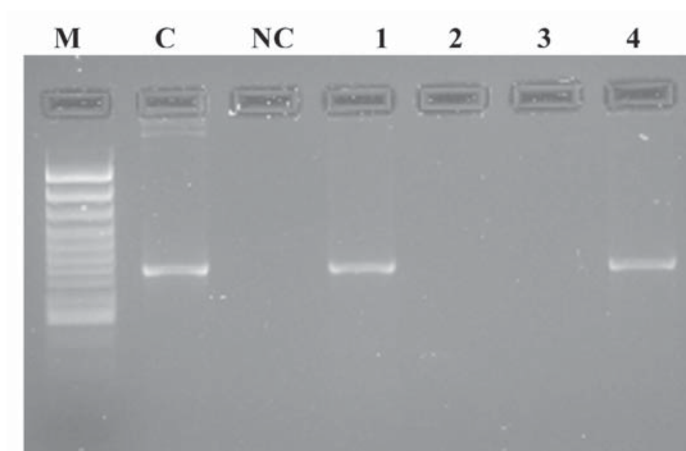


Figure - 2: GFP expression constructs (Cec A Promoter + GFP) detection by PCR in transgenic silkworm. M: Marker, C: Positive control, NC: Negative Control, 1-4: Test samples

AIT 3582 : Development of densovirus resistant productive bivoltine silkworm breeds through marker assisted selection (In Collaboration with CSR&TI, Mysore)

Duration: (Sept. 2016 - Aug. 2018)

Ramesha A, Ponnuvel K.M and Ms. Tania Gupta [JRF]
& Manthira Moorthy [CSR&TI, Mysore]

Flacherie disease is one of the widespread and severe diseases in silkworms, causing up to 20% crop loss. *Bombyx mori* Densonucleosis virus (*Bm*DNV) now designated as *Bm*BDV is one of the major pathogen causing flacherie disease in silkworm. Major symptoms associated with *Bm*DNV infections are flaccid larval body and

diarrhoea, un-spun larvae and turn dark brown in colour and finally die. Other than disinfection, development of disease resistant breeds is a prominent, cheapest and eco-friendly approach to control the disease. Recently, a major gene i.e. *nsd-2* (non-susceptibility to DNV-2) involved in DNV-2 resistance has been mapped and isolated, based on sequence and localization analysis it is proposed to function as a receptor for DNV-2 in the silkworm.

This project aims to identify productive breeds carrying DNV-2 resistant allele and develop DNV-2 resistant productive bivoltine silkworm breeds/hybrids. Previous year we reported screening of 49 productive bivoltine silkworm breeds, identify 29 breeds carrying DNV-2 resistant allele through PCR. Genomic DNA was isolated from the months and two sets of primers were used to identify resistant and susceptible allele of *nsd-2* gene.

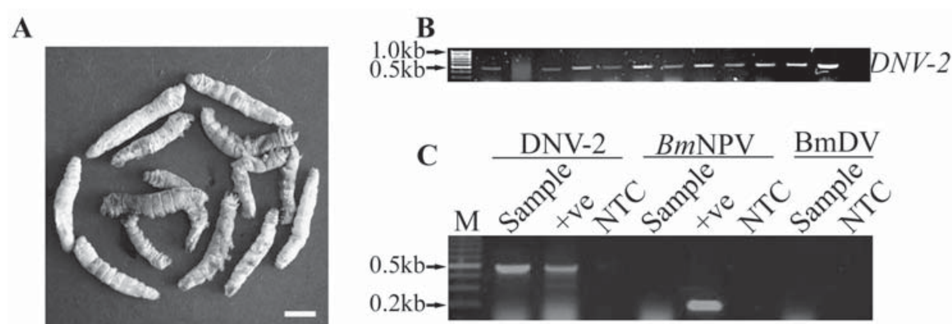


Figure - 3: BmDNV-2 virus inoculum preparation. (A) Silkworms showing typical flacherie disease (B) PCR analysis with VD2 segment of DNV-2 in flacherie diseased silkworms (C) Virus extract prepared contains only BmDNV-2. Genomc DNA from BmDNV-2 resistant and susceptible breeds used as a positive control (+ve) and without genomic DNA used as a no template control (NTC).

DNV-2 virus was isolated from the flacherie diseased silkworm larvae collected from the farmers rearing house in Bangalore rural district. The midgut from diseased silkworm larvae were dissected out and virus extract was prepared and checked for the presence of other pathogens using pathogen specific primers. The result showed that the virus extract contained only DNV-2 and was free from *Bm*NPV and *Bombyx mori* densovirus infections (Figure-3).

Marker assisted selection of CSR6 and CSR26 for DNV-2 resistance

Initial screening revealed CSR6 (10.5%) and CSR26 (23%) of the individuals carry the *nsd-2* resistant allele in homozygous condition and therefore only these percentages of individuals were expected to be resistant to DNV-2 infection. In order to obtain 100%

individuals resistant to DNV-2 infection in CSR6 and CSR26, sib mating was employed wherein the male and female parents were genotyped for *nsd-2* to identify the DFLs carrying homozygous for *nsd-2* resistant allele. These breeds were named as CSR6-R and CSR26-R having *nsd-2* resistant allele in homozygous condition.

The selected newly hatched first instars larvae were infected with DNV-2 through oral feeding. The symptoms started appearing in the susceptible breed (CSR2 and CSR27) around 10 days after infection. The CSR6-R and CSR26-R did not show any symptoms of infection and also, growth and development was observed to be similar to the controls (Figure 4). In case of the susceptible breed, CSR2, none of the infected larvae spun the cocoon while in its corresponding control batch the effective rate of rearing (ERR) was 80%. On the other hand, no significant difference in ERR (%) was observed between the DNV-2 infected CSR6-R and CSR26-R resistant individuals and their corresponding control batches.

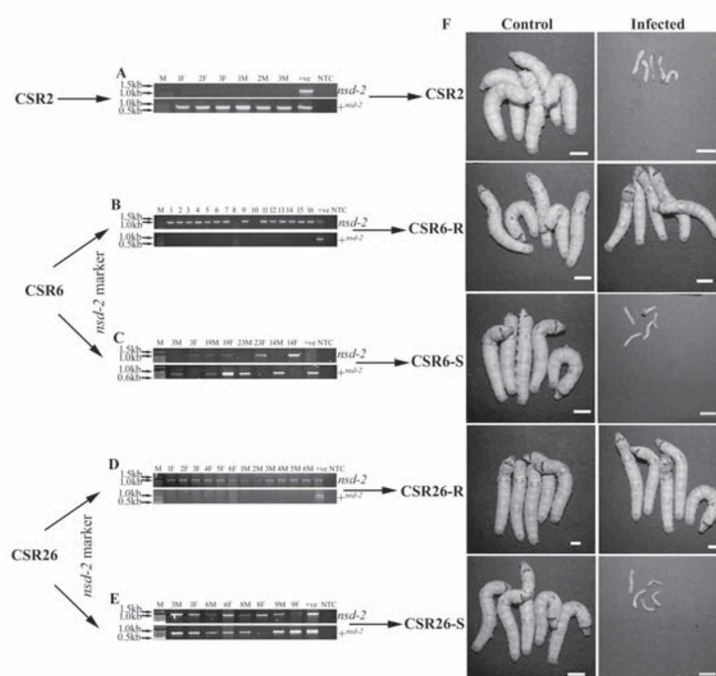


Figure - 4: Selection of BmDNV-2 resistant breeds through MAS and validation of resistance by artificial inoculation. CSR2 breeds carry $+^{nsd-2}$ (Susceptible allele) in homozygous condition (A). *nsd-2* resistant allele as homozygous condition selected in CSR6-R (B). CSR6-S shows $+^{nsd-2}$ in homozygous condition **(C).** Individuals selected carrying *nsd-2* resistant allele in homozygous condition in CSR26-R **(D).** *nsd-2* susceptible allele present in homozygous condition in CSR26-S **(E).** Breeds artificially infected with BmBDV virus validated the resistance selected through functional maker **(F).** Bar = 1 cm

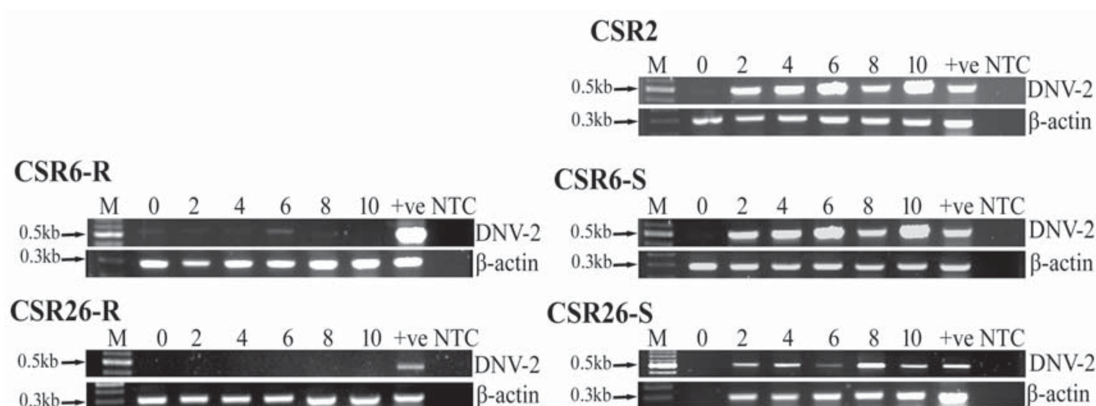


Figure-5: Resistant breeds shows absence of BmDNV-2 (BmBDV infection). PCR analysis using VD2 segment of BmBDV from larvae collected after different days after infection shows presence of BmDNV infection in susceptible breeds (CSR2, CSR6-S and CSR26-S) but not in resistant breeds (CSR6-R and CSR26-R).

In order to test whether CSR6-R and CSR26-R breeds restricted the entry and multiplication of DNV-2, larvae were collected at different days post infection (dpi). Genomic DNA was isolated from the infected larvae and DNV-2 VD2 segment specific primer was used for detecting the DNV-2 infection in the developed resistant breeds. CSR2, susceptible breed showed amplification of DNV-2 when tested at 2, 4, 6, 8 and 10 dpi. However, DNV-2 amplification was not detected in resistant breeds CSR6-R and CSR26-R (Figure-5).

Marker assisted selection of MASN-6 and MASN-7 for DNV-2 resistance

MASN-4, MASN-6 and MASN-7 were developed at Seri-Biotech Research Lab, Kodathi for tolerant to *Bm*NPV were used for screening and selection for DNV-2 resistance. Screening of male and female parents for *nsd-2* resistant allele in MASN lines, selected MASN-6 and MASN-7 carrying *nsd-2* resistant allele in homozygous condition. When these lines were tested for inoculation, MASN-6 and MASN-7performed well compared to MASN-4 and CSR-2, where resistant allele is absent (Figure-6)

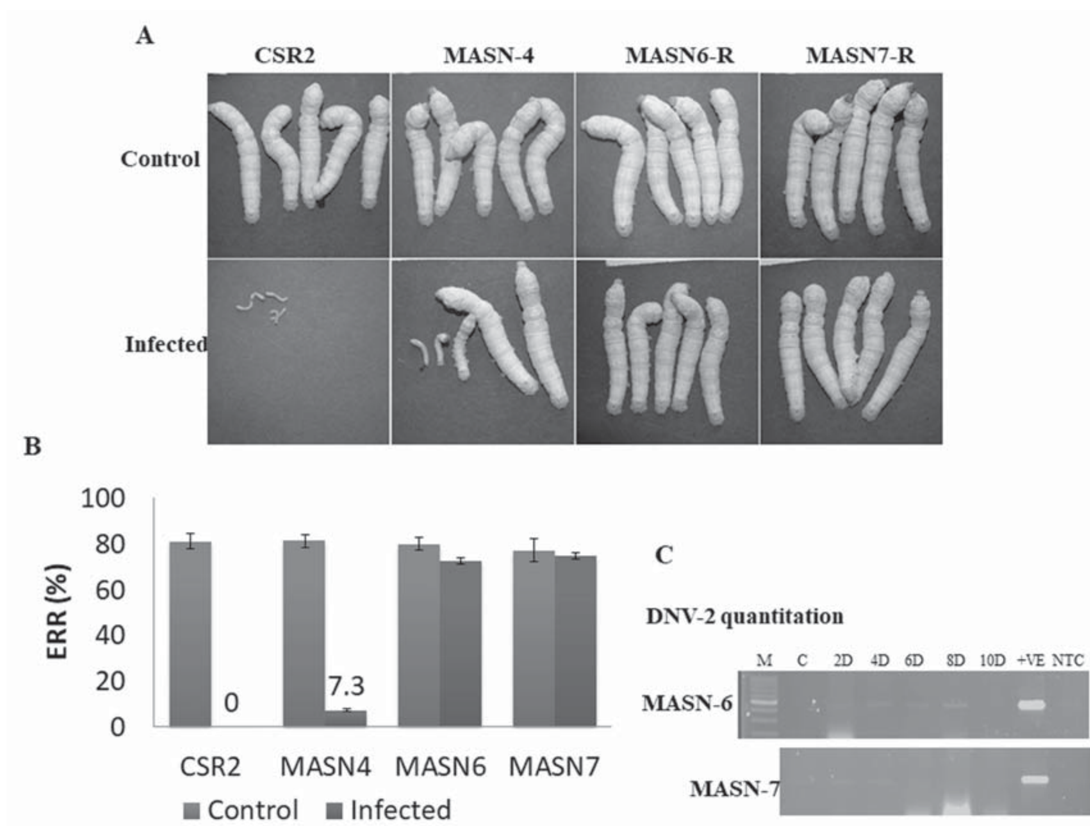


Figure - 6: Validation of MASN lines for BmDNV-2 resistance by artificial inoculation. **(A).** MASN6-R and MASN7-R selected based on *nsd-2* marker shows comparable Effective Rate of Rearing (ERR %) under BmDNV-2 infected condition in comparison with control **(B).** PCR analysis using VD2 segment of BmDNV-2 from larvae collected after different days after infection shows absence of BmDNV-2 infection in resistant breeds, MASN6-R and MASN7-R **(C).**

Conclusions: Results from multiple breeds and generations demonstrated that simple molecular marker, *nsd-2* is sufficient for screening and developing complete resistance against DNV-2 virus infection. Silkworm breeders can easily incorporate *nsd-2* marker in their breeding programme while improving for other economic traits to harness multiple benefits.

AIT 3583 : Trans kingdom RNA interference (tkRNAi) approach for resistance against BmNPV infection in silkworm *Bombyx mori*. L
Duration (Sep 2016- Aug 2019)

Prinicipal investigator: K. S. Tulsi Naik, Co-Investigator: K. M. Ponnuvel
JRF: Ms. Shambhavi Hungund
In Collaboration with Dr. M.V. Rajam, Dept of Genetics,
University of Delhi, South Campus

The efficacy of an RNAi experiment can be influenced strongly by the mode of delivery of the RNAi trigger. Many studies have exploited alternative routes for dsRNA delivery, including electroporation, soaking or ectopic application, incorporation into nanoparticles, expression in bacteria, topical application, injection into woody plants, direct absorption of dsRNA in water solution into plant cuttings, or rooted seedlings and trees and solubilization using transfection agents, such as Lipofectamine .Among all the above methods suggested for delivery of dsRNA into the host. Oral delivery is a less-invasive and potentially a high-throughput method for RNAi delivery. It has particular value for insects that are intolerant of injection and for field applications for RNAi-mediated pest control

Feeding bioassay experiments to validate the effect of dsRNA fed to silkworms

The 50 ml of induced dsRNA *ie-l* and dsRNA *lef-1* and L4440 vector bacterial cells were harvested and was re-suspended in 1ml of autoclaved water to prepare a concentration of 50X. 50µl of bacterial cells was smeared on mulberry leaves cut into small squares. The smeared mulberry leaves were air dried and fed individually to each 5th instar silkworm along with the 2000OBs of BmNPV. After 24 hours of feeding, the mid gut samples were dissected out from virus infected silkworms fed with ds RNA *ie-1* and dsRNA *lef-1* bacterial cells and L4440 vector alone. The genomic DNA as well total RNA was isolated from the mid gut samples. The analysis of genomic DNA with GP41 primers showed presence of virus multiplication in the NPV infected and NPV+L4440 vector treated silkworm mid gut while virus infected silkworms fed with dsRNA *ie-1* and dsRNA *lef-1* bacterial cells showed less virus multiplication. The survivability analysis was analyzed for three batches the results were consistent and showed increase in survivability of dsRNA fed silkworms while NPV infected showed 2-3 % survivability. The survivability was also checked after repeated virus dose at

48 hrs but some of the silkworms did show the symptoms of infection (Figure 7 & 8). Semi-quantitative expression analysis using the cDNA samples of above samples revealed very less expression of *ie-1* gene in NPV+ *ie-1* (dsRNA *ie-1*) and *lef-1* gene in NPV+ *lef-1* (dsRNA *lef-1*) treated silkworms whereas the expression of *ie-1* and *lef-1* gene was higher in the NPV infected and NPV+L4440 vector fed silkworm (Figure-9)

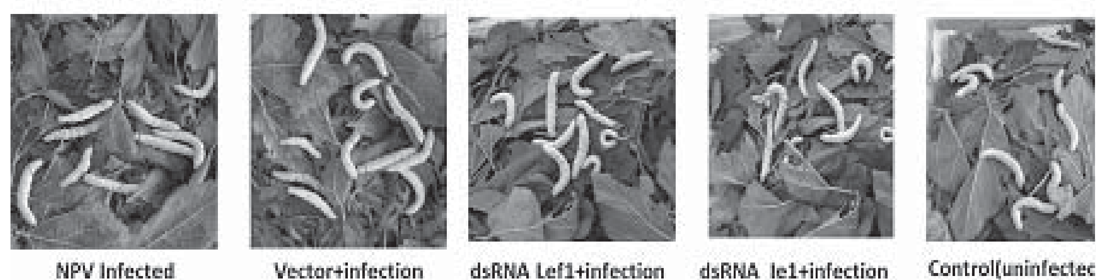


Figure-7: Bioassay experiments showing the effect of dsRNA Lef-1 and dsRNA ie-1 fed BmNPV infected silkworms

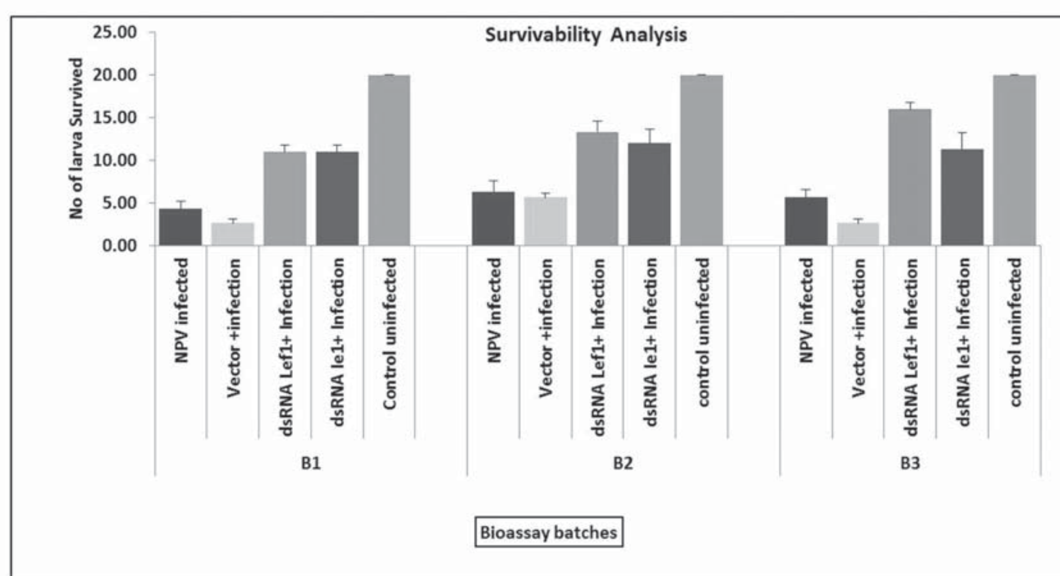


Figure 8: Analysis of the survivability of dsRNA fed BmNPV infected silkworms in three bathes

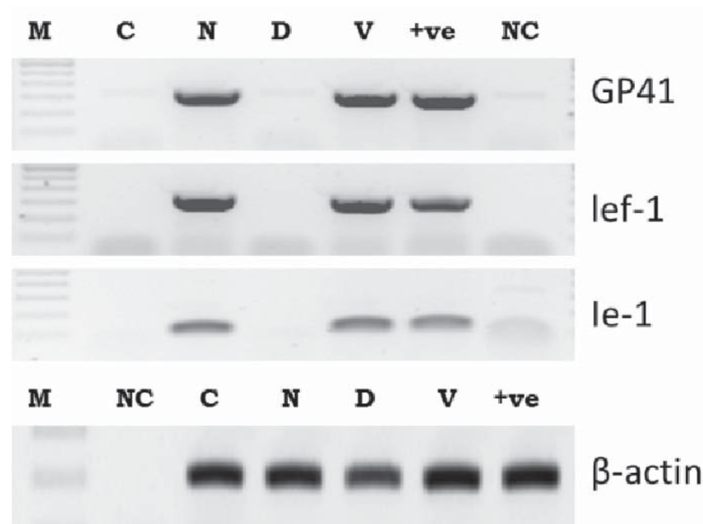


Figure 9: Analysis of viral multiplication using GP41 primers and Semi quantitative analysis of BmNPV viral gene expression in midgut samples of dsRNA fed infected silkworms': marker, C: Uninfected control, N: NPV infected silkworms, D: dsRNA fed infected silkworms, V: L4440 vector fed to infected silkworms, +ve: BmNPV positive, NC: negative control

The results suggest that feeding of *Lef-1* dsRNA at 5th instar silkworms was effective in silencing the *Lef-1* gene expression during BmNPV infection in silkworm. Bacterially expressed dsRNA, when fed to the insect larvae, produced similar effects to other widely used techniques. As the dsRNA is expressed in the bacteria, the degradation is also prevented to a great extent. Further, it was observed that feeding dsRNA is better in terms of specificity and reduced chances of secondary siRNA production in the target organism, which may lead to off-target effects. This study has shown that feeding bacterially expressed dsRNA led to a significant drop in the transcript levels of *ie-1& lef-1* and restricted the viral multiplication in the host as compared to control. Larvae fed with *E.coli* expressing *ie-1* and *lef-1* dsRNA showed a substantial reduction in the expression of target genes involved in the *Bm*NPV multiplication and showed significantly increased the survivability of infected silkworms. Our bio-assay studies revealed that the dsRNA was effective upto 24 -36 hrs. Thus multiple feeds of dsRNA every 24 hours enhances the efficacy of RNAi in the mid-gut of *Bombyx mori.* Overall results indicate 25µg of dsRNA (50µl of bacterial cells) is effective in resisting 2x10^3 spores of BmNPV/larva. Oral delivery of dsRNA is a high-throughput method for RNAi delivery in insects intolerant to injection and for large scale field applications for RNAi-mediated pest 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.*

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)

Duration: **(Sep 2016-Aug 2019)**

K. S. Tulsi Naik and A.R. Pradeep
G. Hariraj [CSTRI, Bangalore] L. Kusuma [CSR&TI, Mysore]
Mrs. Shambhavi Hungund [JRF]
Mr. S. Aravind [JRF]

Every character of an organism is influenced by both hereditary and environmental factors as it is manifested because of long chain interactions of genes either with each other or with the environment. In fact, characters cannot be developed until and unless the genes governing them have proper environment. Heritability of quantitative traits plays very vital role in the selection strategies (Singh et al. 2011 *Advances in Bioscience and Biotechnology*). In any breeding program, information is required about the approximate range of heritability of the traits to be improved and also of genetic correlation among them and with other important characteristics. Currently, modern techniques such as transgenesis and marker-assisted selection are the most effective ways of improving silk properties and they are applied widely in silk production. The advent of molecular marker technology has brought powerful tools for animal and plant breeders to get more markers of animal and plant species. Poly-genic characters, which were very difficult to analyze using traditional animal and plant breeding methods, can be used as molecular markers (DNA markers). Therefore, molecular markers could locate and follow the numerous interacting genes that determine a complex trait. MAS (marker assisted selection) breeding methods could select both phenotypic and genetic traits in molecular in heritance.

A total of 14 QTLs were detected for cocoon filament length, whole cocoon weight, pupal weight, and cocoon filament size. Among them SNPs identified for the filament length was also studied three such SNPs were taken in the present study ie, SNP FL 1 and SNPFL 2 and GFL1 No variations in the size was observed for SNP FL1 and SNP FL2 primers but amplification was observed in all the samples (Figure 10 & Figure 11). In GFL gene amplification two different amplicons were produced by the GFL gene (Figure12) ie, this particular gene showed an amplification of 700bp among the bivoltine race and 600bp among the multivoltine races, therefore this gene was amplified in large quantities from two races separately ie CSR2 and Nistari (multivoltine race) and the

variants showed 100 bp difference in the size of the amplicons. To analyze the GFL1 gene in detail gDNA from selected Multivoltine (PM,Nistari, C.Nichi, Moria, AP12 ,MW13, BL25, TW.SK6.SK1, SK6.SK1.TW) and Bivoltine (CSR2) races were isolated and subjected to PCR amplification with the GFL1 primer, this gene was cloned and sequenced to analyse the sequence among multivoltines and CSR2 (Figure 13). The deletion of 100 bp was observed in all the selected multivoltine races .Further this sequence was subjected to the NCBI BLAST analysis and showed similarity with the Fibroin-H region of *Bombyx Mandarina* (Figure 14). To check the inheritance pattern of this gene segregating population of PM x CSR2 was done to analyze the inheritance pattern of GFL 1 gene. In F1 individuals, PCR amplification was done using GFL1 gene these individuals showed both the amplicons of size 600 bp as well as 700 bp indicating heredity of the gene associated with the filament character and the segregating populations having 600bp,700bp and 600and 700bp amplicons were observed in the F2 individuals indicating inheritance of this gene GFL1 (Figure 15 and Figure-16). Further analysis of this gene and the correlation of this gene with the genes associated with filament characters is in progress.

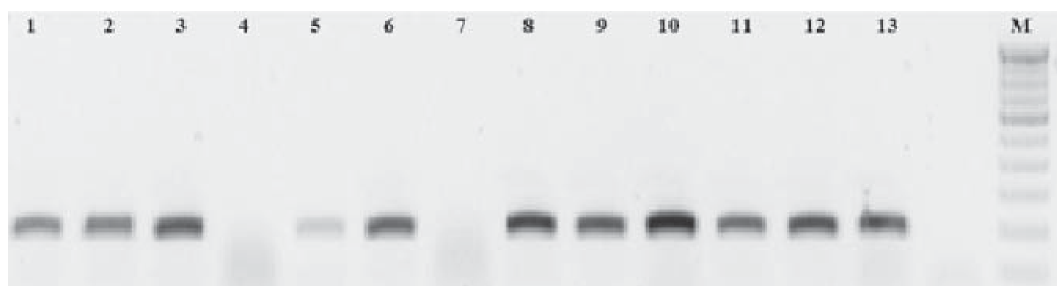


Figure - 10: Genomic DNA Analysis of SNPFL1 gene (210bp) among the selected races. Lane: M– 100 bp DNA Ladder, 1– Pure Mysore, 2– Sarupat, 3– Moria, 4- C.nichi, 5- CSR2, 6- Kolar gold, 7- AP12, 8- MW13, 9- BL24, 10- TW.SK6.SK1, 11- SK6.SK1.TW, 12- Nistari 13- G

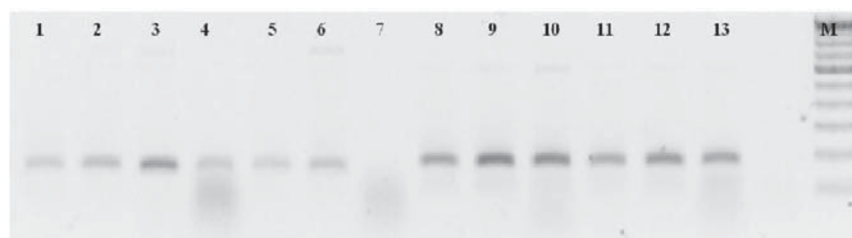


Figure-11: Genomic DNA Analysis of SNPFL 2 gene (200bp) among the selected races. Lane: M– 100 bp DNA Ladder, 1– Pure Mysore, 2– Sarupat, 3– Moria, 4- C.nichi, 5- CSR2, 6- Kolar gold, 7- AP12, 8- MW13, 9- BL24, 10- TW.SK6.SK1, 11- SK6.SK1.TW, 12- Nistari 13- G

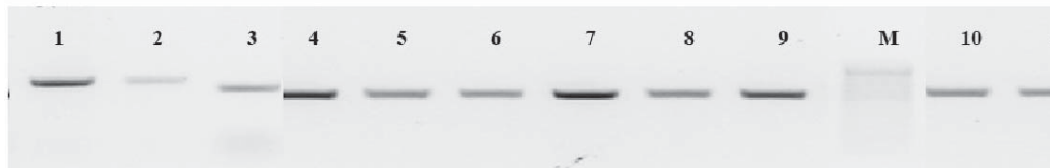


Figure 12: Genomic DNA Analysis of GFL1 gene (600/700bp) among the selected races. Lane: M– 100 bp DNA Ladder, 1– Pure Mysore, 2– Sarupat, 3– Moria, 4- C.nichi, 5- CSR2, 6- Kolar gold, 7- AP12, 8- MW13, 9- BL24, 10- TW.SK6.SK1, 11- SK6.SK1.TW, 12- Nistari 13- G

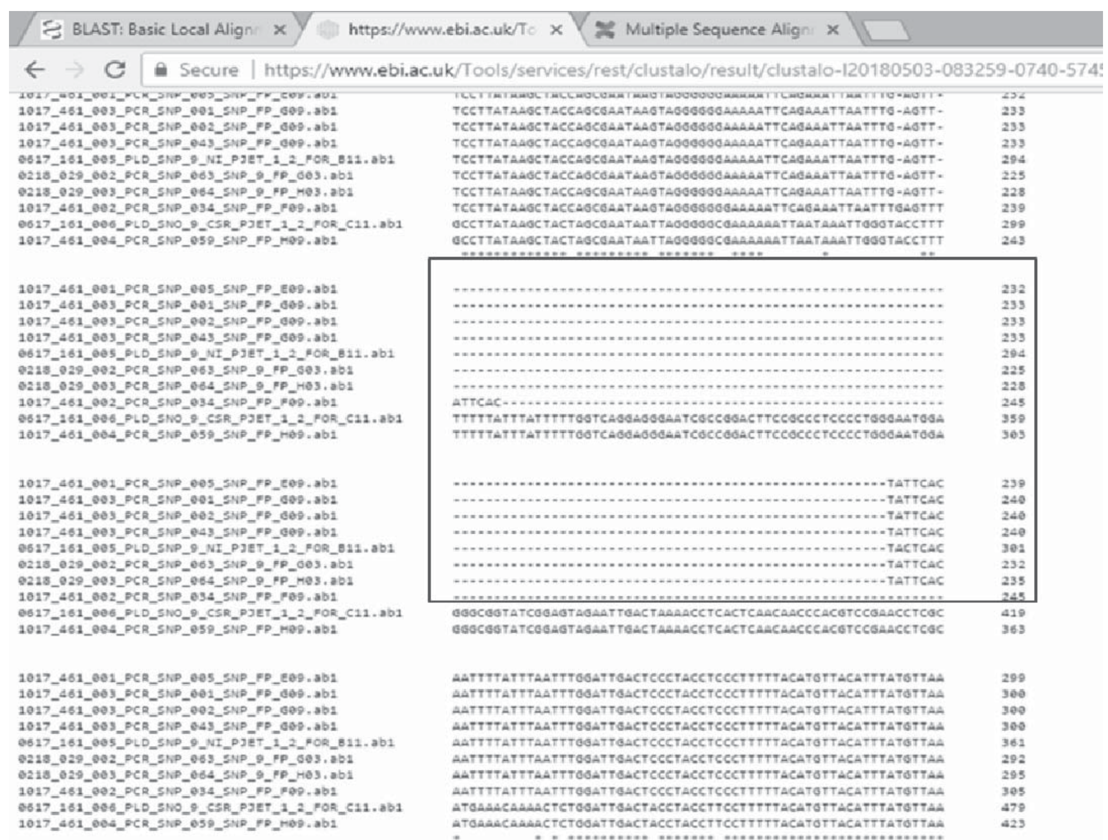


Figure -13: Multiple sequence alignment of GFL 1 gene from selected silkworm races.

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Bombyx mandarina Alps gene for alkaline phosphatase, partial cds	169	169	15%	2e-42	87%	AB379675.1
☐	Bombyx mandarina clone Jmand1 fibroin-H gene, intron	132	132	16%	3e-31	81%	AY441794.1
☐	Bombyx mandarina clone Kmand2 fibroin-H gene, intron	126	126	16%	1e-29	81%	AY441797.1
☐	Bombyx mandarina clone Kmand1 fibroin-H gene, intron	126	126	16%	1e-29	81%	AY441796.1
☐	Bombyx mandarina clone Jmand2 fibroin-H gene, intron	126	126	16%	1e-29	81%	AY441795.1
☐	Bombyx mandarina fibroin gene 5'-end region (exons 1 and 2 partial)	121	121	16%	6e-28	80%	X03973.1

Figure 14: NCBI BLAST analysis of the GFL1 gene sequence

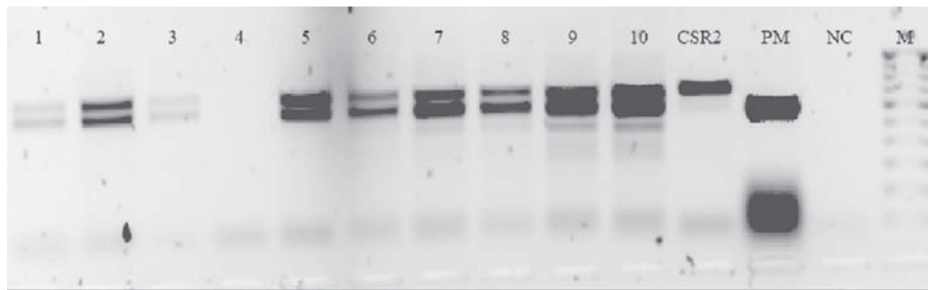


Figure 15: Genomic DNA analysis of F1 Generation (PM X CSR2) using GFL1 primer

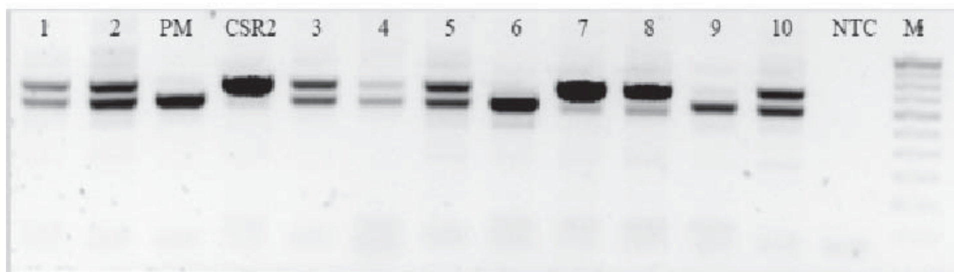


Figure 16: Genomic DNA analysis of F2 Generation (PM X CSR2) using GFL1 primer

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&TIs of Mysore, Berhampore and Pampore and in technical collaboration with NSSO, Bangalore)

Duration: Feb. 2017– Jan. 2020

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

Investigators at SBRL, Bangalore:

Dr. A R Pradeep, Scientist-D , PI

Dr. K M Ponnuvel, Scientist-D, Co-PI

Ms. Sindoorha, [JRF (till Jan 2018), SBRL, Bangalore]

Investigators at CSRTI, Mysore:

Dr. S. Manthira Moorthy, Scientist-D (PI)

Mr. B. Mohan, Scientist-D, SSBS-Coonoor (Co-PI)

Investigators at CSRTI, Berhampore:

Dr. Gopal Chandra Das, Scientist-D (PI)

Dr. N. Chandrakanth, Scientist-B (Co-PI)

Investigators at CSRTI, Pampore:

Dr. Mukesh Tayal, Scientist-D, RSRS-Miransahib, Jammu (PI)

Dr. Mohamed Aslam, Scientist-D, CSRTI, Pampore (Co-PI)

Dr. Pankaj Tiwari, Scientist-D, RSRS-Dehradun, Uttarakhand (PI)

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

1. 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

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

Objectives under 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

Silkworm breeding is aimed mainly at synthesizing new breeds with better NPV tolerance. Molecular marker- assisted selection coupled with conventional breeding techniques had been employed to synthesize new MAS-N lines of silkworm breeds from the cross of Sarupat and CSR2 under technical and research input from CSR&TI, Mysore, KSSR&DI, Bangalore and APSSR&DI, Hindupur. The lines were artificially selected for NPV tolerance and high yield traits for nearly 30 generations and it exhibited better NPV tolerance inherited from Sarupat and high yield traits, from CSR2. Under the standard rearing conditions of 25 ± 2°C, 65-70% relative humidity and 13L:11D cycles employed, the MASN lines showed uniform development and cocoon traits and did not show infection (Figure 17). Under experimental NPV infection conditions, an average of 65 % survival is recorded. Under field conditions, MASN showed approximately 7- 8% infection whereas CSR races showed 20-30% infection showing the sustainability under pathogenic stress. In order to examine the survival and suitability of the MASN lines in different parts of the country, a project has been initiated under DBT funding in collaboration with different sericultural Institutes at Mysore, Berhampore and Pampore.

Figure - 17: Cocoons showing uniformity in size and shape in MASN lines reared at SBRL

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

The three lines developed and reared at different climatic conditions of Coonoor, Jammu, Dehradun and Berhampore. Samples were validated for presence of the DNA markers (Figure 18). Out of the individuals checked, 95% of them were with markers. Those individuals without the markers were rejected. Next generations were developed only from the individuals with the marker. Three markers developed from *deoxy kinase*

(Nag 88), *protein kinase G-1B* (Nag 65) and *glucosyl transferase* (Nag 84) were consistently present in most of the individuals (Fig 2). In addition, new markers viz, *Molybdnum co-sulfurase, Profilin, Lysozyme, Cathepsin, Arylphorin,* stress proteins *Hsp70* and *Hsp90* were screened. *Molybdnum co-sulfurase, Lysozyme* and *Profilin* showed presence in the three MASN lines, *lebocin, cathepsin* and *arylphorin* were present in MASN4 and 6 lines and *Hsp70* and *Hsp90* were present in all the three lines. The PCR profiles of new genes have to be confirmed.

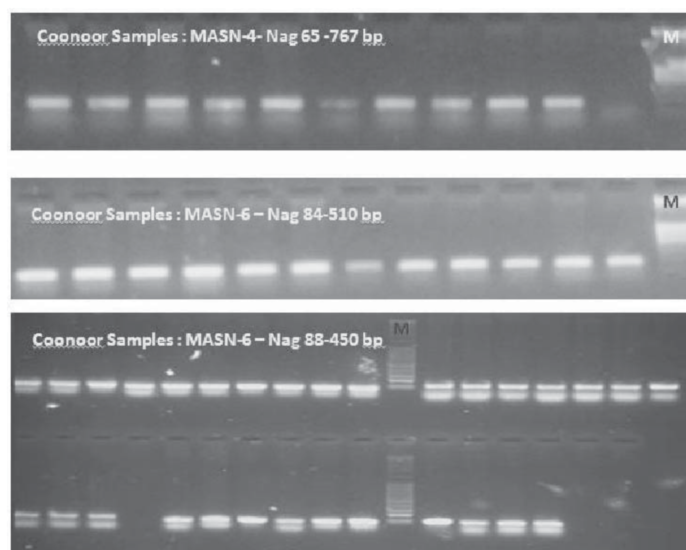


Figure - 18: MASN lines reared at Coonoor showed presence of markers, Nag 65, Nag 84 and Nag 88 associated with NPV tolerance

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

At SBRL, rearing was followed by hatching of eggs treated with HCl as well as using eggs after completion of diapause under preservation schedules. The eggs showed 95 – 99 % uniform hatching in both cases. The fifth instar larvae attained larval weight of 3.4 to 3.6 g. During Nov- December 2017, larvae gained up to 3.9 g to 4.2 g weight. Silk ratio (silk wt / cocoon weight) x 100) reflect the silk content was in the range of 19.5 to 20.85 in summer (May- June 2018) rearing whereas in Nov- Dec rearing it was recorded an average of 22.9% in MASN 6 and 7 indicative of influence of difference in environmental conditions on larval growth and silk production. The average filament length of MASN lines ranged from 721 – 781 meters with 80- 88%

reelability and 84% non-broken filament length (606 - 688 m). These pure MASN6 and MASN7 races showed up to 94% cleanness and 96 % average neatness showing better quality of the filaments. The MASN 7 is graded with '4A' under Major tests and 3A under auxiliary tests. However survival rate of MASN4 and 6 are higher than MASN7 on bioassay with NPV infection under controlled conditions. This showed higher CSR2 traits and probable retention of NPV susceptibility in MASN7 in comparison to MASN 4 and 6, which are balanced races with both higher traits and better survival. The survival rate was upto 95% in June and August rearing while it was reduced to 90-92% in April and November rearing (Figure - 19).

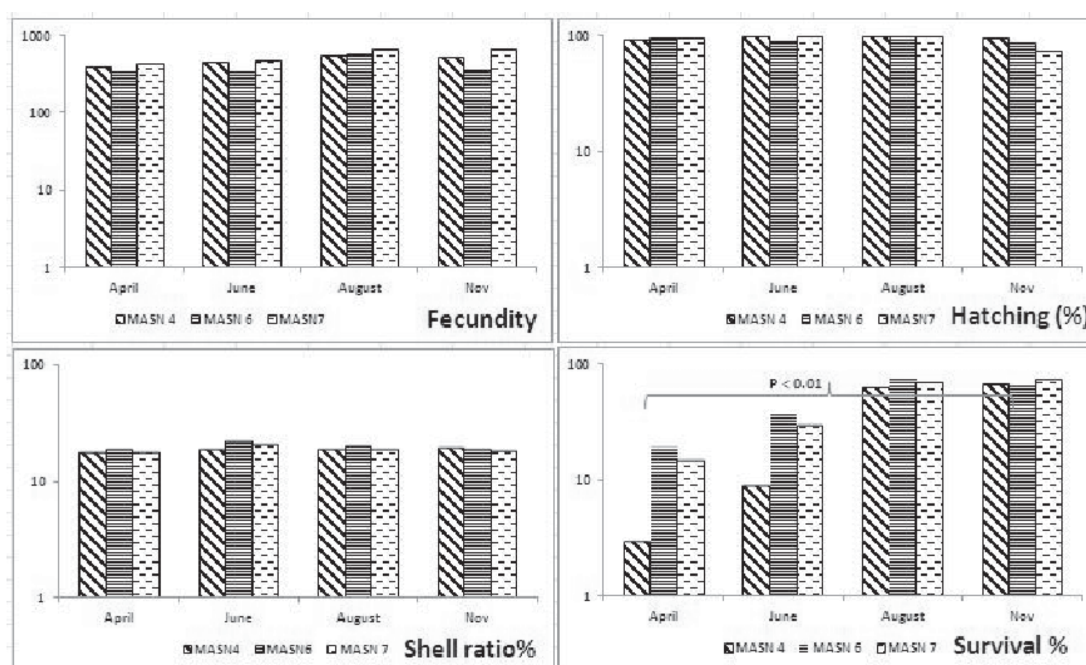


Figure - 19: Log values of fitness and adaptive traits of MASN (SBRL stock) exhibited during rearing in 2017

In order to analyze the cocoon characters and survival rate of bivoltine crosses, MASN x CSR4 (bivoltine x bivoltine) was made and the traits are recorded. Single cocoon weight of MASN x CSR4 hybrids is in the range of 1.8 to 1.9 g and the shell ratio was 20 - 23% (Table 1). Reeling traits were analyzed at CSTRI, Bangalore. The average filament length is 845 to 953 meters out of which non-broken filament length was 797 – 882 meters. The reelability was 92 to 94% with raw silk recovery was in the range of 78 to 85% (**Table 1).** The F1 generation of MASN7 x CSR4 cross showed

99% cleanness, 98% average neatness. Renditta of the breed was in the range of 5.4 to 5.8 which is a major sign of better reeling character. Major tests showed the cocoons of MASN7 as Grade 4A and auxiliary tests showed it as 3A indicating bivoltine hybrids of MASN as high yielders with better NPV tolerance. Multi x Bi crosses and Bi x bi crosses as per objectives are under preparation for further analysis and confirmation.

Table 1: Reeling characteristics of F1 of MASN x CSR4 lines

Traits	MASN-4 x CSR4	MASN-6 x CSR4	MASN-7 x CSR4
CWT	1.839 g	1.847 g	1.944 g
SWT	0.424 g	0.376 g	0.423 g
SR%	23.1 %	20.3 %	21.8 %
Cocoon characteristics			
Average filament length (m)	845 m	865 m	953 m
Non-broken filament length (m)	797 m	813 m	882 m
Single cocoon filament denier	3.54	3.35	3.43
Reeling characteristics			
Reelability	94.3%	94.1 %	92.5 %
Raw silk recovery %	77.8 %	85.2 %	85 %
Silk waste %	12.3 %	11.5 %	9.3 %

MASN-4, MASN-6 & MASN-7 lines were supplied to CSR&TIs of Mysore, Berhampore and Pampore. Rearing has under taken at different seasons in these places and detailed data will be presented in annual reports of the respective Institutes.

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

Investigators: K. M. Ponnuvel – SBRL

S. Subharani and N. Ibotombi Singh – RTRS, Imphal

Duration: Feb 2017 – Jan 2020

In the recent decade, a notorious viral disease commonly known as tiger band disease causes severe damage in oak tasar silk production of *Antheraea proylei* in North east & north west India. This project was aimed to develop DNA based diagnostic tools for early detection of baculovirus causing tiger-band disease. In this direction, the complete viral genome was determined, depicting that the group I alpha baculovirus i.e. *Antheraea proylei* nucleopolyhedrovirus (AnprNPV) is the causal agent of this disease (Figure-20).The full length genome of the virus has now been identified and sequenced (Accession: GI: 1371952746). The analysis of the complete viral genome revealed six conserved regions which were amplified and sequenced. The conserved regions were further found to share homology with alpha-baculovirus (AnprNPV) which is similar to *Antheraea pernyi* NPV (AnpeNPV). Among all the conserved regions the p94 gene segment was chosen for the detection of AnprNPV as it had consistent amplification and was thus a good candidate for the detection of the AnprNPV infection (Figure-21).

Figure - 20: *Antheraea proylei* larvae showing tiger band disease symptoms

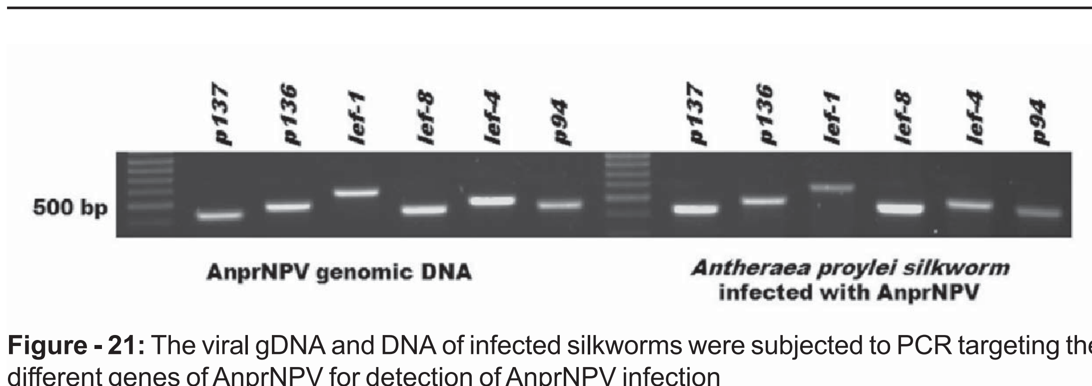


Figure - 21: The viral gDNA and DNA of infected silkworms were subjected to PCR targeting the different genes of AnprNPV for detection of AnprNPV infection

Phylogenetic analysis showed the clustering of AnprNPV in one clade of alphabaculovirus group I of the saturniid silkworm viz. *A. pernyi* and *Philosamia Cynthia ricini* indicating a close relationship among them. The virus particles were detected on silkworm egg surfaces and also in its inner contents. The viral presence was detected and confirmed on surfaces of both washed as well as unwashed eggs, through conventional PCR technique (Figure-22).

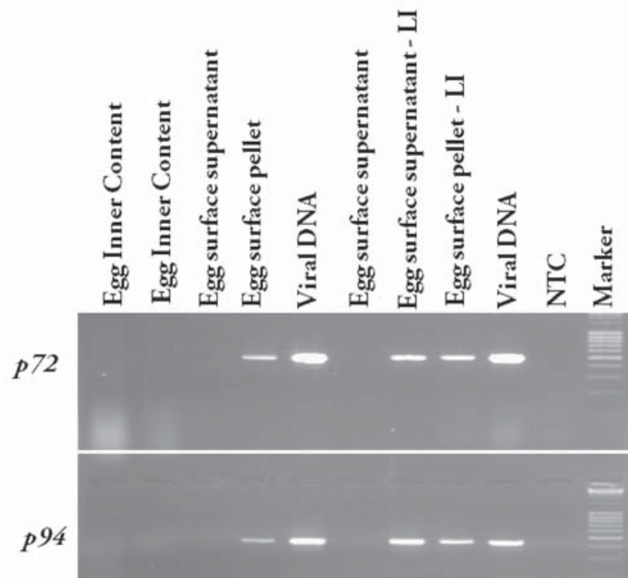


Figure-22: Silkworm egg surface is potential source of virus infection

The presence of virus on egg surfaces thus indicated towards the possible source of infection. The presence of virus particles was also detected at different developmental stages of the silkworm *A. proylei* thereby indicating ubiquitous infection in all tissues (Figure-23).

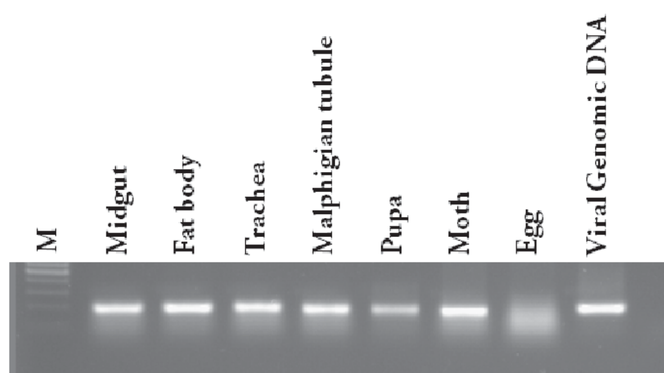


Figure-23: AnprNPV multiplication in different tissues of oak tasar silkworm *A. proylei*

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 virus particles purified were further studied through SEM (Figure-24).

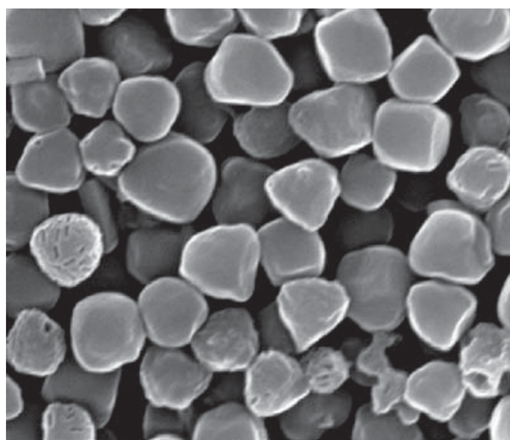


Figure - 24: *Antheraea proyeli* NPV particles purified from infected *A. proyeli* larva

The analysis also led to the identification of other viral pathogens associated with NPV causing tiger band disease in oak tasar silkworms *Antherarea proylei*. One of the prominent association that was detected during this analysis was that of co-infection of Baculovirus and Iflavirus in oak tasar silkworm. The conventional as well as the Real time PCR, both showed amplification for AnprNPV and ApIV, respectively. These results clearly indicate towards the fact that there exists a co-infection between viruses. However, the impact of this coinfection on Baculovirus infection is yet to be analysed.

We also aimed at developing a DNA based diagnostic tool for the early detection of the Tiger Band disease. A Loop-mediated isothermal amplification (LAMP) technique was standardized for easy & simple identification of the virus infection. 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 the infection.

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

Duration: (Oct. 2015 - Sept. 2018)

A. R. Pradeep , K. M. Ponnuvel, Ramesha A. [SBRL]
Kartik Neog [CMERTI, Jorhat], Upendra N. [IISc, Bangalore]
Utpal Bora [IIT Guwahati], Arun Kumar K.P. [CDFD, Hyderabad]
Vijayan K. [CSB]

Indian golden silkmoth or the muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera :Saturniidae) is endemic to north-east region of India. The silkworm produces lustrous golden silk. Due to large public demand, increase in production of muga silkworm and better quality silk is necessity of the silk industry. SSR analysis showed high homozygosity of muga silkworm populations in most of the wild and cultivar (reared) with better heterozygosity in few wild populations Due to the low heterozygosity, *A. assamensis* larvae showed low survival rate and higher susceptibility to pathogens, probably by accumulations of lethal genes due to inbreeding and homozygosity. In the present investigation whole genome sequencing and Transcriptome analysis of male moths of *A. assamensis* were performed. The wild muga cocoons collected from Mokokchung of Nagaland located at a geographical position of (26.6565° N, 94.6399° E) and cocoons of cultivar (reared) muga silkworm larvae were collected from CMERTI, Jorhat. Both wild and cultivar cocoons were emerged and live moths were preserved at -80°C and used for genomic DNA extraction.

Genomic DNA of the male moth of wild and cultivar types of *A. assamensis* was isolated using Blood & Tissue nucleotide isolation kit (Qiagen) and quality check performed using Nanodrop. The whole genome sequencing and the analyses are

performed at a facility available at M/s. Genotypic Technologies Pvt Ltd., Bangalore using Illumina and PacBio platforms.

Four Illumina libraries for short insert shotgun library (SI; 300 bp inserts), long insert shotgun library (LI; 500 bp inserts) as well as mate-pair library of 5-7 Kb insert and 7-10 Kb insert were prepared. The mate pair libraries were prepared following Nextera (Illumina, Austin) matepair (MP) sample preparation kit.

The raw reads and processed reads of both mate pair and PacBio sequences are given in the Table 2. The reads were quality checked using FastQC tool. Data was processed for adapters and low-quality bases (Phred score <Q30 were removed. The SI and LI libraries were processed using Trimgalore for Q30, keeping minimum read length of 50. The MP Libraries were processed using NextClip and Cutadapt tools with cut-off of Q30 and minimum read length of 50.

In order to separate out bacterial genome contamination, the processed paired-end reads SI and LI were mapped to complete bacterial genome database. The bacterial genome contamination was observed to be 0.02 to 0.07 % which were removed from both wild and cultivar samples. PacBIo samples did not show any bacterial contamination.

De Novo assembly of whole genome sequence data

After removal of the low quality reads and bacterial genome, 182842328 Illumina reads and 2386667473 PacBio reads from cultivar and 127880360 Illumina reads and 2062297512 PacBio reads from wild type were assembled using hybrid as well as platform- specific assemblers *viz*., Platanus, Abyss, Soap denovo, minia and MaSuRCA. Low quality reads were replaced using Gapcloser program (Simpson and Durbin 2012) and generated final draft genome.

The reared (cultivar) type has genomic size of ~500 Mb with 2456 scaffolds. For the WT1 sample the assembly resulted in genomic size of ~501 Mb with 2697 scaffolds showing better assembly (Table 3). Size of the genome in *A. assamensis* found larger than the genomes of lepidopterans *Bombyx mori* (432 Mb; International Silkworm Genome Consortium 2008), in wild type, total contig length was 501176205 with maximum length of 4953275 and average length of 185827.3. In cultivar type, total contig length was 500314777 with maximum length of 3198103 and average length of 203711.2. Analysis on Genome characteristic is under progress.

Table 2: Read statistics of Illumina pair end and mate pair ends and coverage during whole genome sequencing of *A. assamensis*

Sample Name	Raw Reads	Processed Reads	Chemistry	Platform	Coverage (X)
SO-6793-Set2-Muga-C1-Ext2-short-insert	85825377	81646036	151*2	Illumina HiSeq	56.34
SO-6793-Set2-Muga-C1-Ext2-long-insert	87923333	82585545	151*2	Illumina HiSeq	57.72
SO-6793-Set2-Muga-C1-Ext2-MP-7-10kb	49388292	14233488	151*2	Illumina HiSeq	32.42
SO-6793-Set2-Muga-C1-Ext2-MP-5-7kb	10481159	3524239	151*2	Illumina HiSeq	6.22
SO-6793-Set2-Muga-C1-Ext2-MP-5-7kb	14114786	853020	75*2	Illumina NexSeq	4.18
SO-6793-Muga-WT1-Male-Short-Insert	30085334	28734265	151*2	Illumina HiSeq	19.75
SO-6793-Muga-WT1-Male-Long-Insert	69963188	64801925	151*2	Illumina HiSeq	45.93
SO-6793-Re-Ext-2-Muga-WT1-Male-MP-5-7kb	51976974	21355830	151*2	Illumina HiSeq	34.12
SO-6793-Re-Ext-2-Muga-WT1-Male-MP-7-10kb	36127155	12988340	151*2	Illumina HiSeq	23.71

Note : In Table 1 Read counts shows single end representation (either R1 or R2)

Table 2. Pacbio Read Statistics

Sample Name	Total Reads	Total Read Length	Coverage
SO-6793-Set-2-Muga-C1	234308	2386667473	4.76
SO-6793-Muga-WT1-Male	227694	2062297512	4.11

Table-3: Assembly statistics of C1 and WT1 sample

	WT1	C1
Contigs Generated :	2,697	2456
Maximum Contig Length :	4953275	3198103
Minimum Contig Length :	1,000	1001
Average Contig Length :	185827.3	203711.2
Median Contig Length :	89743	125465
Total Contigs Length :	501176205	500314777
Total Number of Non-ATGC Characters :	4185318	1484676
Percentage of Non-ATGC Characters :	0.835	0.297
Contigs >= 100 bp :	2,697	2456
Contigs >= 200 bp :	2,697	2456
Contigs >= 500 bp :	2,697	2456
Contigs >= 1 Kbp :	2,697	2456
Contigs >= 10 Kbp :	1,749	1750
Contigs >= 1 Mbp :	113	115
N50 value :	683,227	661913

C1 – cultivar (reared); WT1- wild type

CONCLUDED RESEARCH PROJECTS

ARP 3518 : Expression profiling of genes associated with resistance to *Beauvaria bassiana* in *Bombyx mori* silkworm strains (In Collaboration with CSGRC Hosur)

Duration: **Oct. 2014 - Sep. 2017**

Geetha N. Murthy and K.M. Ponnuvel
P. Somasundaram and N. Balachandran [CSGRC, Hosur]

In order to decipher the correlation of antifungal gene expression profile variations with fungal proliferation, an analysis of the rate of proliferation of *B.bassiana* spores in the selected silkworm breeds was carried out through qPCR wherein the *B.bassiana* gene copy numbers were compared among the breeds. The qPCR results indicated that, the *B.bassiana* gene copy numbers were lesser in MV breeds compared to BV which is in concurrence with the fact that, MV breeds are known to be hardy compared to BV. The lower fungal proliferation in the MV breeds APDR15 and Nistari as well as in the BV breeds The high *B.bassiana* gene copy numbers in MV breeds MH1 and *C.nichi* as well as in CSR4 and CSR27 indicate that, these breeds could be less tolerant to the fungus.

Reports suggest that, tolerance /susceptibility to fungi is based on the levels of certain saturated fatty acids like caprylic capric and linoleic acids in the epiculticular layer of silkworm integument that inhibit invasion of the fungi. Higher amounts of these fatty acids on the cuticle inhibit the invasion of fungi. Thus it can be inferred that, the aforesaid fatty acid levels could be lower in the breeds with higher copy numbers of *B.bassiana* gene as it reflects higher proliferation of the fungus in these breeds. The higher copy numbers of *B.bassiana* gene in multivoltine breeds indicates their tolerance to fungal infection attributed to the distribution of such fatty acid chains in the epicuticular layers.

In general, our study revealed that most of the antifungal genes expressed less in uninfected (control) samples, while, the expression levels increased after *B.bassiana* infection in the breeds in which fungal proliferation was less. Further, among the different silkworm breeds, MV breeds revealed higher expression of genes on *B.bassiana* infection compared to BV breeds. Susceptibility to muscardine disease varies among different silkworm breeds with MV breeds being usually less susceptible than the BV breeds. Experimental studies conducted in this line of research at CSR&TI, Mysore, India resulted in the identification of one multivoltine (MV1) and one bivoltine

(BV1) as fungal resistant out of 52 and 40 silkworm stocks screened through fungal infections, respectively (Sudhakar Rao *et al*., 2011). In the present study many breeds revealed drastic reduction of antifungal gene expressions in infected breeds along with higher proliferation of the fungus. Thus the overall results indicate that, these antifungal genes are a line of defense against fungal infection but at the same time express only on attainment of a particular level of infection which triggers their expression activating the Toll pathway to control rate of infection.

Output of the study

❖ 14 multivoltine [MV] and 14 bivoltine [BV] silkworm breeds were selected for the study based on various traits such as highly productive, popular, native, exotic, robust as well as low performing.

❖ Based on semi-quantitative expression analysis of 14 antifungal genes in the selected silkworm breeds, 5 antifungal genes with consistent differential expression were shortlisted for real time PCR [qPCR] expression analysis to screen the silkworm breeds.

❖ Five antifungal genes *viz.* Neutral lipase, Amidase, Glucose transporter, Arylphorin, and Gloverin were shortlisted for screening MV and five *viz.* Glucose transporter, Amidase, Gloverin, Arylphorin, Lysozyme for BV breeds.

❖ Among the MV breeds, two antifungal genes Amidase and Arylphorin revealed constant higher expression in control as well as in infected samples while other three genes Glucose transporter, Neutral lipase and Gloverin showed lower expression after infection. Among the BV breeds, four antifungal genes Amidase, Gloverin, Glucose transporter and Lysozyme, showed constant increase in the antifungal gene expression both in control and after infection, while, Arylphorin gene showed lesser gene expression in all the breeds after infection.

❖ The MV breeds APDR15 (highly productive) and Nistari (Popular) indicated lower fungal multiplication with higher expression of the antifungal genes. In contrast *C. nichi* (Popular) and MH1 (highly productive) showed higher fungal multiplication with lower expression of the antifungal genes. Among BV breeds A and J2 (P) (autumn top performing) followed by APS5 and APS4 (robust breeds), CSR27 and CSR 4 (popular) and Barupat (low performer) revealed higher expressions of the genes in treated compared to control. Thus the MV breeds APDR15 and *Nistari* and BV breeds J2(P) and A indicated tolerance to fungal infection, while, the MV

breeds *C. nichi* and MH1 and BV breeds CSR 4 and CSR 27 indicated susceptibility to fungal infection.

❖ The overall outcome indicates that, fungal multiplication is less in MV breeds compared to BV breeds indicating that the latter are susceptible to fungal infections. Hence, MV breeds are best suited for tropical climate as they are much tolerant to fungal infections.

Utilization of the outcome:

Among the multivoltine races Nistari and APDR15 and among the bivoltine races A and J(2)p are much tolerant to muscardine disease that are evident from the lower fungal multiplication and from the study it was found that among the antifungal genes that were selected for the study Amidase, Arylphorin and glucose transporter gene expression were higher in both infected and uninfected tissue samples. They can be utilized further as a potential markers for screening the germplasm to identify the races that are tolerant to muscardine disease and theses races can be further utilized in the breeding program to develop disease resistant/tolerant races.

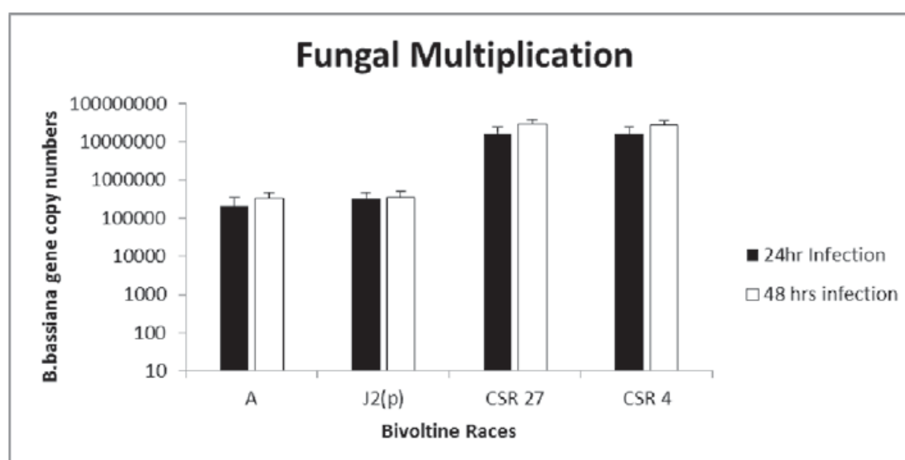


Figure-25: qPCR analysis of the Copy number of *Beauveria bassiana* gene in 24 hrs and 48 hrs infected integument samples of shortlisted multivoltine races

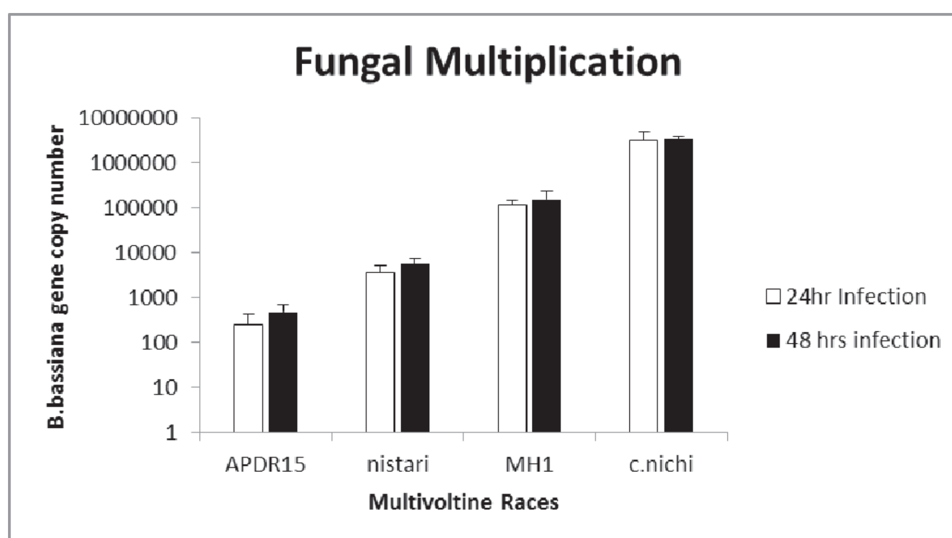


Figure 26 : qPCR analysis of the Copy number of *Beauveria bassiana* gene in of 24hrs and 48 hrs infected integument samples of shortlisted bivoltine races

Silkworm Stock maintenance

1. Six lines of Transgenic CSR4 and CSR27
2. Silkworm breeds for NPV resistance developed through marker assisted selection, MASN4, MASN6 and MASN7.
3. 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 / Conference / Workshops

As part of capacity building program in this institute, 18 Post graduate and Engineering students belonging to different colleges were given 1-6 months hands-on training in the molecular biology techniques.

RAC Meeting

Research Advisory Committee meeting was held on 5th September 2017 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
OFFICIAL LANGUAGE IMPLEMENTATION

<table>
<tr><th>क्र.सं.</th><th colspan="2">राजभाषा कार्यान्वयन</th><th>''घ'' क्षेत्र में हिंदी पत्रचार में प्रास लक्ष (%)</th><th>टिप्पिणी प्राप्त लक्ष</th></tr>
<tr><td>1.</td><td rowspan="4">तिमाही प्रगति</td><td>अप्रैल-जून तिमाही</td><td>56.4</td><td>40.0</td></tr>
<tr><td>2.</td><td>जुलाई-सितम्बर</td><td>56.8</td><td>45.8</td></tr>
<tr><td>3.</td><td>अक्टूबर-दिसंबर</td><td>67.2</td><td>56.0</td></tr>
<tr><td>4.</td><td>जनवरी-मार्च</td><td>62.0</td><td>72.3</td></tr>
<tr><td></td><td colspan="2">कार्यशाला का आयोजन</td><td colspan="2">19.06.2017
28.09.2017
18.12.2017
16.02.2018</td></tr>
<tr><td></td><td colspan="4">राजभाषा में उल्लेखनीय कार्य / उपलब्धियों</td></tr>
<tr><td>1.</td><td colspan="4">SBRL वेबसाइट पूर्ण रूप से हिंदी में बनया गया</td></tr>
<tr><td>2.</td><td colspan="4">रेशम जैव प्रौद्योगिकी अनुसन्धान प्रयोगशाला से निस्त्रत प्रौध्योगिकी हिंदी तकनीकी Phamplet बनाया गया है</td></tr>
<tr><td>3.</td><td colspan="4">सरकारी कामकाज मूल रूप से हिंदी में करने के लिए
1) डॉ के एस तुलसी नायक, वैज्ञानिक-बि को प्रथम पुरस्कार (10697 शब्द)
2) श्री चन्द्रशेकर राव, सहायक अधीक्षक को द्वितीय पुरस्कार (5270 शब्द)
3) श्री मोहन राजू सहायक अधीक्षक को तृतीय पुरस्कार (4695 शब्द)</td></tr>
<tr><td>4.</td><td colspan="4">हिंदी पखवाडा
हिंदी पखवाडा दिनांक 14-28 सितम्बर को आयोजन किया गया था इस पखवाडा में सभी वैज्ञानिकों / कर्मचारियों को कई प्रतियोगिताएं का आयोजन भी किया गया था</td></tr>
</table>

PUBLICATIONS

1. Gupta T, Ito K, Kadono-Okuda K, Murthy GN, Gowri EV and Ponnuvel KM (2017) Characterization and genome comparison of an Indian isolate of bidensovirus infecting the silkworm *Bombyx mori*. **Archives of Virology.** https://doi:10.1007/s00705-017-3584-x

2. Tulsi Naik K.S., Ponnuvel K.M. and Awasthi A.K. (2017) Transkingdom RNA interference approach to improve resistance against Grasserie disease in *Bombyx Mori*.L –A Review. **Sericologia** 57 (1) 1-9

3. Varada B, Pradeep AR, Awasthi AK, Sivaprasad V, Ponnuvel KM and Mishra RK (2017) Non-target Host Immune Gene Modulation in Transgenic Silkworm *Bombyx mori* Endowed with RNAi Silence *Bm*NPV Genes. ***Biotechnology Journal International (British Biotechnology Journal)*** 20(4): 1-12, 2017; Article no.BJI.39442 ISSN: 2456-7051

4. Pooja M, Pradeep AR, Hungund SP, Sagar C, Ponnuvel KM, Awasthi AK and Trivedy K (2017b) Oxidative stress and cytotoxicity elicited lipid peroxidation in hemocytes of Bombyx mori larva infested with dipteran parasitoid, *Exorista bombycis*. **Acta Parasitologica,** 2017, 62(4), 000–000; ISSN 1230-2821. DOI: 10.1515/ap-2017-00.

5. Gourab Roy, Kalidas Mandal and G. Ravikumar (2017) PCR-based detection of microsporidia in silkworms using non coventional RNA polymerase primers. **Biosci.Biotech.Res.Comm.**10:676-679.

6. Shambhavi PH, Pradeep A R and Mandal K (2018) Microsporidian infection in *Bombyx mori*: Molecular Challenges" paper presented at **National Conference on Seri-Biomics: Challenges, Innovations and Solutions at University of Mysore** (Feb 15 – 17, 2018)

7. Tanya Gupta, Ramesha A, Ponnuvel KM, Mandal K and Moorthy MS (2018) Genetic variation in resistance to BmBDV vurus and its utilization in development of disease resistant productive silkworm breeds. Paper presented at **National Conference on Seri-Biomics: Challenges, Innovations and Solutions at University of Mysore** (Feb 15 – 17, 2018)

8. Ravikumar G and Mandal K (2018) Vertebrate low density lipoprotein receptor homologue from eri silkmoth *Samia ricini*. **8th International Conference on Wild silkmoths, 22-24 January 2018, Guwahati.**

9. Ponnuvel KM, Ito K, Terenius O, Miranda J de, Jayaprakash P and Mandal K (2018) Genome sequence, characterization and quantification of a novel Ifla Virus infecting the tropical tasar silkworm, *Antheraea mylitta*. **8th International Conference on Wild silkmoths, 22-24 January 2018, Guwahati.**

10. Technical bulletin on translational technologies developed by SBRL **in Hindi**.

ii Seminar/ workshop / Trainings attended

1. **Dr Tulsi Naik and Dr K M Ponnuvel**- Workshop on "Next generation sequencing and data analysis using Bioinformatics Tools"- National Symposium On future of Functional Genomics at Transdisciplinary University Bangalore from 13-14 October 2017

2. **Dr Tulsi Naik and Dr A Ramesha -** Orientation Training On Usage Of Windowstat packages(Ver-9.2) at CSGRC, Hosur on 9-10th November 2017.

3. **Dr Tulsi Naik-** Visited Delhi University South Campus Dept Of Genetics for 10 days to carry out collaborative work on Cloning and expression of dsRNA in bacteria to elicit RNA interference against BmNPV in silkworms.

4. **Dr Ravikumar G and Dr KM Ponnuvel** attended the International Conference on Wild silkmoths at Guwahati, India (January 2018).

5. **Dr A R Pradeep and Dr A Ramesha-** attended National Conference on Seri-Biomics: Challenges, Innovations and Solutions at University of Mysore (Feb 15 – 17, 2018)

6. **Dr. Tulsi Naik, Dr Ramesha A and Dr A R Pradeep** attended the Seri-breeders meet on 20- 21st February, 2018 at CSB, Bangalore

Innovations from ongoing projects

1. Establishment of silkworm transgenic facility and over-expression of immune genes through transgenesis

2. Silk fibroin H-cecropin B fusion protein is expressed and purified in *Pichia pastoris* using Pichia pink expression system

3. Identification of virus that causes tiger band disease: Morphological and gene expression analysis led to identification of baculovirus as the causative organism for Tiger band disease in the Indian oak tasar silkworm *Antheraea proyeli*.

New breeds for field testing

1. Lines developed for NPV tolerance through marker assisted selection, MASN-4, MASN-6 and MASN-7 are maintained and tested for presence of markers and confirmed it. These lines are sent to Jammu RSRS, Dehradun RSRS and CSRTI at Mysore and Berhampore. Season-wise variation in survival rate is recorded with higher survival of up to 90% is recorded at Jammu in April-May 2017 rearing whereas in Berhampore, highest survival of 70% was recorded in October-November 2017 rearing. At Bangalore, rearing was successful with 95-97% survival throughout the year. Under CSRTI, Mysore rearing at RSRS Kodathi, Salem, Chamarajnagar and at Coonoor was successfully conducted in favourable seasons. Further testing is under progress.

ACKNOWLEDGEMENTS

SBRL would like to acknowledge the following organizations/Institutes for their support and co-ordination in R&D activities of this laboratory

Sl. No	Name of the Institute
1.	Department of Biotechnology, Government of India
2.	Department of Science & Technology, Government of India
3.	IISc, Bangalore
4.	IIT, Guwahati
5.	Dept. Of Genetics, University of Delhi , South Campus
6.	CDFD, Hyderabad
7.	APSSRDI, Hindupur
8.	M/s Unilever Industries, Bangalore
9.	C-CAMP, Bangalore
10.	NIMHANS, Bangalore
11.	SSTL, Kodathi, Bangalore
12.	RSRS, Kodathi, Bangalore
13.	NSSO, Bangalore
14.	CSTRI, Bangalore
15.	CSR&TI, Mysore
16.	CSR&TI, Berhampore, West Bengal
17.	CSGRC, Hosur
18.	CTR&TI, Ranchi
19.	CMER&TI, Lahdoigarh
20.	CSR&TI, Pampore
21.	BTSSO, Bilaspur

RESEARCH ADVISORY COMMITTEE

Sl.No.	List of RAC Members	Remarks
1.	**Prof. P.N. Rangarajan** Dept. of Biochemistry, Indian Institute of Science Bangalore – 560 012	**Chairperson**
2	**Prof. Upendra Nongthomba** Dept. of Molecular Reproduction and Developmental Genetics Indian Institute of Science Bangalore – 560 012	**Member**
3	**Prof. Malali Gowda** Professor and Group Head, Centre for Functional Genomics and Bioinformatics Transdisciplinary University, Yelahanka, Bangalore	**Member**
4	**Prof. Anitha Peter** Professor, Dept of Plant Biotechnology, UAS, GKVK, Bangalore - 560 065	**Member**
14	**The Director** Seri-Biotech Research Laboratory Kodathi, Carmelram post, Sarjapur Road Bangalore - 560035	**Member-Convener**

INSTITUTE BIO SAFETY COMMITTEE

Sl No	Name	Address	Remarks
1	Prof. Usha Vijayaraghavan	Department of Microbiology & Cell Biology, Indian Institute of Science, Bangalore -12	Member-Secretary
2	The Director	Seri Biotech Research Laboratory, Bangalore - 35	Chairman
3	Dr. R. Ashokan	Principal Scientist, Dept. of Biotechnology IIHR, Hesaraghatta Bangalore - 560 089	Member
4	Dr. B. L. Mohan Kumar MBBS	AMA-CSB Employees Dr. Mohan Nursing Home Sarjapura Road, Bangalore - 35.	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.K. Awasthi	Scientist D & PI Seribiotech Research Laboratory,	Member Internal Expert
8	Dr. A. R.Pradeep	Scientist D & CI Seribiotech Research Laboratory, Bangalore - 35	Member Internal Expert

VISITORS

Dr. Ranjith Ranjan Okhandiar, IFS, Member Secretary of Central Silk Board visited SBRL, Kodathi and interacted with all the Scientists, research fellows and Staff of SBRL regarding the major research activities and requirements of the laboratory. He appreciated the research activities of SBRL and advised to undertake translational work that would benefit the stake holders

Japanese Scientists, Dr. Shuichiro Tomita and Dr Natuo Komoto, Instt of Agrobiological Sciences, National Agricultural & Food Research Organization, Tsukuba, Japan visited the lab on 28 January, 2018 and presented lecture on transgenic silkworms and its utility

HUMAN RESOURCES

Sl.No.	Name	Designation
1	Dr. Kalidas Mandal	Director (retired on 28.02.2018)
2	Dr. R. K. Mishra	Director (since 01.03.2018)
3	Dr. K. M. Ponnuvel	Scientist- D
4	Dr. G. Ravikumar	Scientist- D
5	Dr. A. R. Pradeep	Scientist- D
6	Dr. K. S. Tulsi Naik	Scientist- B
7	Dr A. Ramesha	Scientist- B
8	Mr. S. N. Gundurao	Technical Assistant
9	Mr. G. Sumant Kumar	Technical Assistant
10	Mr. Srikantaiah	Technical Assistant

RESEARCH FELLOWS / ASSISTANTS

Sl. No	Name	Designation	Division
1	Mr. Wazid Hussain	Research Associate (CSIR)	Proteomics
2	Mrs. Varada Burdekar	Senior Research Fellow	Genomics
3	Ms. Tania Gupta	Junior Research Fellow	Genomics
4	Mrs. Vijaya Gowri	Junior Research Fellow	Genomics
5	Ms. Shambhavi P Hungund	Junior Research Fellow	Proteomics
6	Mrs. Dyna Susan Thomas	Junior Research Fellow	Transgenic/ silk biomaterial
7	Mrs. Chitra Manoharan	Junior Research Fellow	Transgenic/ silk biomaterial
8	Mrs. Sandhya Rasalkar	Junior Research Fellow	Transgenic/ silk biomaterial
9	Ms. Shruti M	Junior Research Fellow	Genomics
10	Ms. Aarti	Project Assistant	Genomics
11	Ms. Shinthura Raman	Junior Research Fellow	Genomics/ Proteomics
12	Mr. Naresh Kumar	Rearing Assistant	Genomics/ Proteomics

ADMINISTRATION

Sl No	Name	Designation
1	Mrs. Shyamala Murthy	Asst. Director
2	Mr. Mohan Raj	Asst. Supreintendent
3	Mr ChandrashekharRao	Asst. Supreintendent
4	Mr A. Mallesha	Asst. Technician
5	Mr. S. Nagesh	Driver
6	Mr.Kenchappa	Multi tasking Staff

SUPPORTING STAFF

Sl No	Name	Designation
1	Mr. L.P Sampangi Rao	SFW
2	Mr. Hombalaiah	SFW
3	Mr. M. Krishnappa	SFW
4	Mr. N Pillappa	SFW
5	Mr. E Shivanna	SFW
6	Mrs.Lakshmamma	TSFW

SUPERANNUATION

Dr. Kalidas Mandal, Director, SBRL retired from service of Central Silk Board (Government of India) on attaining superannuation at the age of 60 years on 28th February 2018. His major contributions to sericulture industry included sericulture extension and silkworm egg production. The fraternity of SBRL thanking him for his esteemed service and wish him a happy retired life.

FINANCIAL PROGRESS (2017-18)

During the year under report, an expenditure of Rs. 215.96 lakhs was incurred from Grants-in-Aid sanctioned by CSB and Department of Biotechnology, Government of India

S.No.	Particulars	Amount (Rs. In lakhs)
1	Salary and allowances	133.63
2	Wages and EPF	14.13
3	Travelling Expenses	1.25
4	Contingent Expenses	35.04
5	Assets	14.33
6	Others	1.41
7	**Sub Total**	**199.79**
8	DBT Assistance	16.17
9	**Total**	**215.96**

Other Events

Vigilance Awareness week

Vigilance Awareness week was organized from 30th October to 4th November 2017 as per instruction of Government of India and Central Silk Board. A debate competition on the theme “My vision: Corruption free India” was also conducted to create awareness about corruption free India

Swacchta Pakwada

Swacchta pakwada was organised from 01.03.2018-15.03.2018 jointly with Silkworm Seed technology laboratory, (SSTL), Kodathi and various programme was organised as per central office instructions.