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प्रक्कथन

भारत में रेशम उत्पादन ग्रामीण अर्थव्यवस्था के विकास से काफी निकटता से जुड़ा हुआ है। रेशम कृषकों और धागाकरण / बुनाई समुदाय इसके सहायक स्तंभ है, जिन्हें रोग मुक्त रेशमकीट पालन, रेशम उत्पादन और इसके उपयोग के लिए प्रोत्साहित किया जाना आवश्यक है। समग्र रेशम उत्पादन की वृदधि हेतु प्रारंभिक रोग का पता लगाने के लिए प्रविधियों का विकास, रेशम कीट की सुरक्षा और बेहतर गुणवता के रेशम का उत्पादन कुछ ऐसे कारक हैं जो एक महत्वपूर्ण भूमिका निभाते हैं। इस लक्ष्य को प्राप्त करने के लिए, परंपरागात और आण्विक प्रजनन, बीमारी सहिष्णुता के लिए मार्कर का चयन, रेशम के बायोमेडिकल अनुप्रयोगों पर नए दृष्टिकोण, रोगजनकों का पता लगाने और इसके नियंत्रण, जीनोमिक लक्षण वर्णन और ट्रांसक्रिप्टोम विश्लेषण आदि के लिए इस प्रयोगशाला में कुछ जैव-तकनीकी दृष्टिकोण अपनाए गए हैं। प्रयोगशाला ने विभिन्न शोध कार्यक्रमों के लिए भारतीय-जापानी सहयोग और जैव प्रौदयो्गिकी विभाग (नई दिल्ली) के माध्यम से केन्द्रीय रेशम बोर्ड, विज्ञान और प्रौदयोगिकी विभाग (भारत सरकार) से वित्तीय सहायता प्राप्त की है और हम हमेशा उनके इस समर्थन के ऋणी रहेंगे।

वर्ष 2016-17 के दौरान इस प्रयोगशाला ने तीन परियोजनाओं को अंतिम रूप दिया और पांच नई शोध परियोजनाओं की शुरुआत की और चल रही परियोजनाओं को जारी रखा। एक प्रमुख भारतीय पृथक्कारी वायरल रोगज़नक़, बीएमडीएनवी (बीएमबीडीवी) को पूरी तरह अनुक्रमित किया गया और इसके फाईलोजनी ने अपने जापानी पृथकता के साथ करीबी समरूपता दिखायी है। उत्पादक प्रजाति एपीएस-5 और बीबीई198 में एनएसडी-2 जीन की अभिव्यक्ति ने पुष्टि की कि रेशमकीट *बॉबेक्स मोरी* के उच्च उपज वाले उत्पादक अभिगमों में डीएनवीवी सहिष्णुता होती है्। इसके अलीवा, डेनसॉवायरस-2 प्रतिरोधी जीन वहन करने वाली 30 दविप्रज प्रजातियों का चयन भी समयुग्मजी या विषमयुग्मजी दशा में किया गया। उच्च उर्वरता और प्रजनन क्षमता के साथ रेशम कीट को पहचानने के प्रयास में, रेशम कीट प्रजातियों की जांच हेतु विटेल्लोजेनिन रिसेप्टर जीन का चयन मार्कर के रूप में किया जा सकता है। *बी मोरी* के प्रतिरक्षात्मक तंत्र की जांच करने पर, तरल लैक्टिन और एरिलफोरिन जैसे नए लक्ष्य अणुओं को यूजीमक्खी और लघुबीजाणु संक्रमण के निकट सानिध्य में पहचाना गया, जिसका उपयोग बी मोरी की प्रतिरक्षी प्रजातियों की पहचान के लिए किया जाएगा तथा जिसे भविष्य में और रोग प्रतिरोधक क्षमता के विकास हेतु आगामी प्रजनन कार्यक्रम में इस्तेमाल किया जा सकता है।

एनपावी संक्रमण पूरे भारत में रेशम उदयोग की फसल-हानि का प्रमुख कारण है। एनपीवी सहिष्णुता के बड़े स्तर के साथ उच्च उपज देने् वाली प्रजातियों के विकास हेतु, पारंपरिक प्रजनन और आरएनए हस्तक्षेप प्रौदयोगिकी का एकीकरण किया गया है। सीएसआर 4 और सीएसआर 27 जैसी उच्च रेशम पैदावार वाली प्रजातियों को प्रयोगशाला स्तर पर पारजीनी नि्स्तरी और सीएसआर प्रजातियों के साथ पारंपरिक बैक क्रॉस प्रजनन दवारा बढ़ी एनपीवी सहिष्णुता के साथ पारजीनी वंशों में बदल दिया गया। दूसरी तरफ एनएपीवी संक्रमण को नियंत्रित करने के लिए प्रयोगशाला स्तर पर ट्रांसकिंगडम आरएनएआई तंत्र की शुरुआत की गई है तथा बेहतर तंतु लक्षणों के साथ रेशम कीट प्रजातियों

के चयन हेतु तंतु लक्षणों से संबदध आण्विक मार्करों की पहचान भी शुरू की गई है। रेशम कृषकों की सहायता के लिए, आण्विक मार्कर सहायता-प्राप्त चयन दवारा एनपीवी सहिष्णुता के साथ नई उच्च उपज वाली नस्लों का विकास किया गया और उसे इस वर्ष से विभिन्न भौगोलिक और कृषि-जलवायु परिस्थितियों में केरेबो-केरेअवप्रसं के सहयोग से डीबीटी की वित्तीय सहायता के साथ कृषि परीक्षण के अंतर्गत रखा गया है। आवश्यक आण्विक हस्तक्षेप का एक अन्य क्षेत्र उत्तर-पूर्व भारत और हिमालयी क्षेत्र में ओक तसर रेशमकीट का भारी संक्रमण है जहां टाइगर बैंड रोग ने ओक तसर कृषि का नाश कर दिया है। इम्फाल में केतअवप्रसं-क्षेतअके के सहयोग से डीबीटी की वित्तीय सहायता के साथ आण्विक साधनों के जरिए रोगजनकों की पहचान चल रही है।

रेशम के जीवचिकित्सा अनुप्रयोगों के इस्तेमाल पर, सूक्ष्मजैविक विरोधी गुणों के साथ फाइब्रॉइन प्रोटीन विकसित करने के लिए पारजीनी के साथ संलयन प्रोटीन प्रौदयोगिकी के उपयोग से नए तरीकों का विकास किया जा रहा है। मूगा रेशमकीट, *एंथेरिया असमेन्सिस*, के जीवाण्विक संक्रमण और रेशम गुणों से जुड़े प्रकार्यात्मक जीन की पहचान करने के लिए विभिन्न राष्ट्रीय संस्थानों के साथ मिलकर पूरे जीनोम अनुक्रमण तथा विभिन्न ऊतकों का अनुलेख्य विश्लेषण प्रगति पर है।

सामान्य तौर पर हमने रेशम-जैवप्रौदयोगिकी के सीमांत क्षेत्रों में चुनौतीपूर्ण शोध परियोजनाओं को लिया हैं जिसकी प्रासंगिकता सीधे रेशम उत्पादन के विकास से है। इसके अलावा, प्रयोगशाला विभिन्न स्नातकोतर परियोजनाओं के माध्यम से कई संस्थानैं के वैज्ञानिकों और विभिन्न विश्वविदयालयों के छात्रों को प्रशिक्षण दे रही है।

मैं भारत सरकार की विभिन्न योजनाओं और कार्यक्रमों को लागू करने के लिए प्रशासनिक शाखा के अथक प्रयासों की सराहना करता हूं। विभिन्न शोध परियोजनाओं को लागू करने में सभी शोध छात्रों और वैज्ञानिकों के बहुमूल्य सहयोग के प्रति आभार व्यक्त करता हूँ और मैं उन छात्रों को बधाई देता हूं जिन्होंने वर्ष 2016-17 के दौरान डॉक्टरेट डिग्री प्राप्त की।

इस अवसर पर मैं विभिन्न राष्ट्रीय और अंतर्राष्ट्रीय अनुसंधान संगठनों का भी आभार व्यक्त करना चाहूंगा जिन्होंने प्रत्याक्ष या परोक्ष रूप से हमारे अनुसंधान क्षेत्र में सहायता प्रदान की।

मैं आने वाले वर्षों में वार्षिक रिपोर्ट में सुधार के लिए रचानात्माक आलोचना का स्वागत करता हूं।

डॉ. कालिदास मण्डल
निदेशक

FOREWORD

In India, silk production is closely associated with development of the rural economy. Sericultural farmers and the reeling / weaving community are the supporting stem, who are needed to be encouraged towards disease-free silkworm rearing, silk production and its utilization. In order to enhance the overall silk production, development of methodologies for early pathogen detection, silkworm protection and production of improved quality silk are some of the factors that will play a pivotal role. In order to achieve the said goals, conventional and molecular breeding, marker assisted selection for disease tolerance, newer approaches on biomedical applications of silk, detection of pathogens and its control, genomic characterization and transcriptome analysis are the few biotechnological approaches followed in this laboratory. The laboratory received financial assistance from Central Silk Board, Department of Science & Technology (Government of India) through Indo-Japanese collaboration and Department of Biotechnology (New Delhi) for various research programs and we always owe it to them for the financial and scientific support.

Under 2016-17, this laboratory concluded three major research projects and initiated five new ones and implemented the ongoing projects. Indian isolate of a major viral pathogen, *Bm*DNV (or BmBDV) is completely sequenced and its phylogeny showed close homology with its Japanese isolate. Expression of *nsd-2* gene in productive races APS-5 and BBE 198 confirmed the DNV tolerance in high yielding productive accessions of the silkworm *Bombyx mori*. In addition, 30 bivoltine breeds carrying densovirus-2 resistance gene either in homozygous or heterozygous condition were also identified. In an attempt to identify silkworm races with high fecundity and fertility, vitellogenin receptor gene could be identified as a marker for screening silkworm races. On investigating the immunogenic mechanism of *B. mori*, new target molecules such as humoral lectin and arylphorin were identified in close association with uzifly and microsporidian infection which will be used to identify immunocompetent races of *B. mori* that can be used further in the future breeding programs for developing disease resistance. Identification of functional gene markers associated with fungal infection in *B. mori* needs to be applied in furthering breeding programs including tolerance to fungal attack.

NPV infection is the major cause for crop loss for the sericulture industry, throughout India. In order to develop high yielding races with larger level of NPV tolerance, an amalgamation of conventional breeding and RNA interference technology has been used.

High silk yielding races such as CSR4 and CSR27 were transformed to transgenic lines with larger NPV tolerance by conventional back cross breeding between transgenic Nistari and CSR races at laboratory level. On the other hand, transkingdom RNAi mechanism has been initiated at laboratory level to control the NPV infection. In order to identify molecular markers associated with the filament characters molecular approach has been initiated to identify the silkworm races with better filament characters. Towards assistance to sericulture farmers, new high yielding breeds with larger NPV tolerance are developed by molecular marker- assisted selection and they are under farm trials from this year at different geographical and agro-climatic conditions through collaboration with CSB-CSR&TIs with financial assistance from DBT. Another area of urgent molecular intervention is the drastic infection of oak tasar silkworm in the north- east India and Himalayan belt where tiger band disease has wiped out oak tasar culture. Identification of the pathogen through molecular means is under progress with DBT funding in collaboration with CTR&TI- RTRS at Imphal.

On the utilization of biomedical applications of silk, development of new approaches using fusion protein technology coupled with transgenesis is under progress to develop fibroin protein with anti- microbial properties. In order to identify functional genes associated with bacterial infection and silk properties of muga silkworm, *Antheraea assamensis*, whole genome sequencing and Transcriptome analysis of different tissues are under progress in collaboration with different national institutes.

In general we have taken up challenging research projects in frontier areas of Seri-biotechnology that has direct relevance with sericulture development. Moreover, the laboratory is giving training to scientists of different institutes and students of different universities through post graduate projects.

I appreciate the administrative wing for their untiring effort in implementing various schemes and programs of Government of India. Valuable contribution of all research fellows and scientists in implementing different research projects is acknowledged and I congratulate the scholars who received the Doctoral Degree during 2016-17.

In this occasion I would also like to thank the various National and International research organizations that supported our area of research directly or indirectly.

I welcome the constructive criticism on this edition of annual report for improvement.

Dr. KALIDAS MANDAL
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

वर्ष 2016-17 की मुख्य विशेषताऐं

1. एनपीवी पारजीनों के अंतर्गमन तथा पारंपरिक पश्च संकरण द्धारा उच्च उत्पादक सीएसआर4 तथा सीएसआर27 पारजीनी प्रजातियों में परिवर्तित किया गया। बीसी4एफ16-एफ18 पीढ़ी के पारजीनी वंशों से पता चला कि एनपीवी संक्रमण के उपरांत सामान्य सीएसआर वंशों के 12૩ की अपेक्षा इसमें 56૩ की उत्तरजीविता थी। सीएसआर4 तथा सीएसआर27 के सामान्य शुद्ध वंशो की तुलना में एफ16 तथा एफ18 पीढ़ी के पारजीनी वंशों में डिम्भकीय तथा प्यूपा भार में काफी वृद्धि थी। शुद्ध सीएसआर प्रजातियों की तुलना में पारजीनी वंशों में कोसा विशेषकों की दृष्टि से उचिच एनपीवी सपिष्णुता तथा उसा सीएसआर पारजीनी वंशों में उत्पादन विशेषता के गुण थे।2

. ऊजी मक्खी (*एक्सोरिस्टा बॉम्बीसिस*) के ग्रसन से प्रतिक्रियात्मक ऑक्सीजन प्रजातियों में वृद्धि प्रेरित हुई, जो रेशमकीट, *बॉम्बिक्स मोरी* की रक्त कोशिका में कोशिका विष प्रेरित किया और प्रतिरक्षित प्रतिक्रियाओं को दबाया जिससे परजीवी उत्तरजीविता संभव हुई। बी सेरी डिम्भक में लघुबीजाणु (नोसिमा बॉम्बीसिस) के संक्रमण के बाद, टोल मार्ग सक्रियण में देरी हुई, संभवत: यह टोल मार्ग को सक्रिय करने के लिए बीजाणु के द्धार स्तर की आवश्यकता के चलते हुआ, जिससे बीजाणु की प्रारंभिक उत्तरजीविता और प्रगुणान संभव हुआ। उल्लेखनीय है कि मेलेनाइजेशन घटनाक्रम बाद के दिनों में दब जाता है, जो परपोषी रेशमकीट डिम्भक में संक्रमण के पश्चात वाले दिनों में प्रगुणन और सफल उत्तरजीविता को और आगे समर्थन देता है। सृजित जानकारी का उपयोग करते हुए, रेशमकीट *बॉम्बिक्स मोरी* के प्रतिरक्षी सक्षम विभेदों की पहचान जननद्रव्य से की जाएगी और नए प्रतिरक्षी सक्षम और उच्च उपज देने वाले विभेदों को संश्तेषित करने के लिए प्रजनन कार्यक्रमों का उपयोग किया जाएगा।

3. रेशम प्रोटीन, फाइब्रॉइन, सेक्रोपिन बी और फाइब्रॉइन-सेक्रोपिन संलयन प्रोटीन के चिकित्सा अनुप्रयोगों के लिए जैव निम्रीकरणीय घाव ठीक करने वाले प्रोटीन विकसित करने के लिए इसे व्यक्त कर पता लगाया गया और शुद्ध भी किया गया। जर्मलाइन परिवर्तन के लिए इन प्रीटीनों की एन्कोडिंग करने वाले जीन के साथ बैक्टर, अंडे में सूक्ष्मइंजेक्शन के लिए तैयार है।

4. रेशमकीट की प्रतिरक्षा बढ़ाने के लिए और सूक्ष्म जैविक रोगजनकों के सापेक्ष अधिक प्रतिरक्षा के साथ रेशमकीटों के वंश विकसित करने के लिए, तीन प्रतिरक्षा संबंधी जीन, जैसे *रीलिश*, सेक्रोपिन बी और *ड्रोसोमाइसिन* और उनके प्रमोटरों को सूअर बैक वैक्टर में क्लोन किया गया और डीएनए अनुक्रमण द्वारा इसकी पुष्टि की गई। जीन या इससे संबंधित प्रमोटरों में कोई परिवर्तन नहीं हुआ, जिसकी पुष्टि अनुक्रमण से की गई। प्रतिरक्षा को बढ़ाने के लिए प्रतिरक्षा प्रोटीनों के अत्यधिक विषमता के लिए वेक्टर का इंजेक्शन दिया जाएगा।

5. एसडीएस-पीएजीई विश्लेषण और द्रव्य स्पेक्ट्रमतिति द्वारा *स्टेफिलाकोकस* विटूलिनस जीवाणु संक्रमण के बाद एकत्रित रेशमकीट हीमोलिम्फ प्रोटीन के विश्लेषण से जीवाणुरोधी प्रोटीन जैसे ग्लोवरिन और लाइसोसाइम में काफी संख्या में पेप्टाइड्स् की उपस्थिति देखी गई।

6. कवक ब्यूवेरिया जाति द्वारा संक्रमण के वाद प्रतिरक्षा जीन सक्रियण की पहचान करने के लिए पांच द्विप्रज नस्लों के डिम्भकीय अध्यावरण के नमूने, संक्रमण के 24 घंटों के बाद एकव किए गए। अध्यावरण नमूने से कूल आरएनए निकाले गए। पांच प्रतिकवक जीन, जैसे अमिडेस, अरिल्फोरिन, ग्लूकोस ट्रांसपोटर ग्लोवेरिन और ल्य्सोज्यम अर्द्ध-परिमाणात्मक अभिव्यक्ति विश्लेषण में विभेदक अभिव्यक्ति दर्शाये। वास्तविक समय विष्लेषण के आधार पर दो

अलग-अलग प्रजातियाँ जैव-आमापन के लिए और प्रत्तिकवक जीन अभिव्यक्ति और कवक प्रगुणन दर के बीच संबंध विश्लेषण के लिए चुने गए। चयनित जीन का उपयोग रेशमकीटों की स्क्रीनिंग के लिए किया जाएगा ताकि उन्हें फफूंद सहिष्णुता के साथ रेशमकीट नस्लों के संश्लेषण के लिए पैतृक के रूप में इस्तेमाल किया जा सके।

7. *बॉम्बिक्स मोरी* में बीएमएनपीवी संक्रमण के प्रतिरोध सुधार हेतु, एक नया रोग प्रबंधन अभिगम 'ट्रांसकिंग्डम आरएनए इंटरफरेंस' प्रारंभ किया गया है। बीएमएनपीवी संक्रमण में शामिल छह एनपीवी जीन अर्थात् 1, *लेफ1, लेफ3, पी74, पी35 और पी143* की अनुक्रमण जानकारी क्लोनिंग और ब्लास्ट विश्लेषण के माध्यम मे प्राप्त की गई है। इसके अलावा इन जीनों को एल 4440 वेक्टर के रूप में क्लोन किया गया है और दो जीन अर्यात् 1 और लेफ1 के लिए डीएसआरएनए उत्पादन शुरू किया गया है।

8. रेशम तंतु लक्षणों से जुडे आण्विक मार्करों की पहचान और इन आण्विक मार्करों का उपयोग बहुप्रज प्रजातियों में सुधार के लिए, कार्यात्मक जीन मार्कर-संबद्ध विश्लेषण शुरू किया गया है। तंतु लभणों के साथ जुड़े कई जीनों की पहचान विभिन्न ट्रांसस्क्रिप्टोम विश्लेषण से की गई। फेगो अवाधि के दौरान अलग-अलग समय पर चयनित बहुप्रज अभिगमों के रेशम ग्रंथियों के विभिान्न क्षेत्रों में बारह जीनों का अभिव्यक्ति- विश्लेषण किया गया। 12 जीनों में से, सेरीन ग्लाइसीन चयापचय मार्ग, फाइब्रॉइन-एच और फाइब्राँइन-एल जीन से जुड़े तीन जीनों ने यह दर्शाया कि अर्द्ध मात्रात्मक पीसीआर द्वारा जीन अभिव्यक्ति में भिन्नता थी।

9. डीएनवी-प्रतिरोधी और अतिसंवेदनशील मार्करों की उपस्थिति के आधार पर डेन्सोवायरस प्रतिरोधी-द्वि-संकर के विकास के लिए, फाउंडेशन क्राँस के पैतृक हेतु स्क्रीनिंग शुरू की गई। बीबीई 266 और सीएसआर 6 की पहचान, द्विसंकर विकसित करने के लिए पैतृक के रूप में की जाती है और वर्तमान में बीबीई266 का उपयोग सीएसआर2 और सीएसआर27 के साथ पश्च संकर के विकास हेतु दाता अभिभावक के रूप में किया जाता है। डेनसाँवायरस-2 प्रतिरोध जीन वहन करने वाले 30 द्विप्रज नस्ल भी समयुग्मजी या विषमयुग्मजी दशा में पहचाने गये।

10. अधिक संख्या में उच्च गुणवत्ता के अंडे बनाने वाले अभिगमों की पहचान हेतु वीजीआर जीन का इस्तेमाल एक कार्यात्मक मार्कर के रूप में किया जा सकता है। उच्च वीजीआर प्रतिलेख स्तर वाली उच्च प्रजातियां सकारात्मक सहसंबंध दिखाते हुए बड़ी संख्या में अंड- उत्पादन के लिए जानी जाती हैं, इसका उपयोग उच्च जनन-क्षमता के चयन के लिए आशाजनक रेशम कीट प्रजातियों की सक्रीनिंग में एक कार्यात्मक मार्कर के रूप में किया जा सकता है।

11. पूर्ण लंबाई के भारतीय पृथक्कारी जीनोम बीएमबीजीवी को (एनसीबीआई अभिाम संख्या केएक्स760110 और केएक्य 779526) अनुक्रमित किया गया। फाइलोचेनेटिक और अनुक्रम विश्लेषण से पता चला कि बीएमबीजीवी के भारतीय पृथक्कारी की समजातता जापानी पृथक्कारी बीएमबीडीवी के काफी करीब है।

12. अभिव्यक्ति और संक्रमण के बीच संबंध: एनएसडी-2 जीन के अभिव्यक्ति अध्ययन के लिए चयनित रेशम कीट प्रजातियों से बीएमबीडीवी संक्रमित मध्यांत्र ऊतक के नमूनों को लिया नाया। उत्पादक प्रजाति एपीएस-5 और बीबीई198 अपने विषाणु प्रतिरोधी लक्षणों की पुष्टि के साथ न्यूनतम अभिव्यक्ति दिखायी। पोलीमरेज जीन की अभिव्यक्ति का संबंध विभिन्न रेशम उत्पादन क्षेत्रों से एकत्र संक्रमित रेशम कीट में विषाणु भार मे स्थापित किया गया।

RESEARCH HIGHLIGHTS

1. High yielding CSR4 and CSR27 races were transformed to transgenic races by introgression of NPV transgenes and conventional back crossing. The transgenic lines of BC4F16 – F18 generations showed 56% survival rate after NPV infection in comparison to 12% in infected control CSR lines. In F17 and F18 generation of transgenic lines there was significant increase in larva and pupa weight as well as cocoon traits which are nearing to pure lines of CSR4 and CSR27, thereby attained higher NPV tolerance and yield traits in the same transgenic CSR lines.

2. Uzifly (*Exorista bombycis*) infestation induced increase in Reactive oxygen species which induced cytotoxicity in hemocytes of the silkworm, *Bombyx mori* and suppressed immune responses enabling the parasitic survival. After infection with microsporidian (*Nosema bombycis*) spores in *B. mori* larva, Toll pathway activation is delayed, probably due to requirement of a threshold level of spores to activate toll pathway which enabled initial survival and multiplication of the spores. Notably, melanization events are suppressed in the later days which further supported the multiplication and successful survival of microsporidian in the later days of infection in the host silkworm larva. Utilizing the information generated, immunocompetent strains of the silkworm, *Bombyx mori* will be identified from the germplasm and will be utilized for breeding programs to synthesize new immunocompetent and high yielding strains.

3. In order to develop biodegradable wound healing protein for medical applications of silk protein, Fibroin, Cecropin B and fibroin-Cecropin fusion proteins were expressed, detected and purified. The vectors with the genes encoding these proteins are ready for microinjection into eggs for germline transformation.

4. In order to increase the immunity of silkworm and develop silkworm lines with larger immunity against microbial pathogens, three immune associated genes viz *Relish, Cecropin B* and *Drosomycin* and their promoters were cloned in piggy Bac vectors and is confirmed by DNA sequencing. There were no mutations either in the genes or its respective promoters which was confirmed by sequencing. The vector will be injected for overexpression of the immune proteins to increase the immunity.

5. Analysis of silkworm haemolymph proteins collected after *Staphylococcus vitulinus* bacterial infection on cross breed of *B. mori,* M.com4 x B.com4 by SDS-PAGE analysis and mass Spectrometry showed presence of significant number of peptides of antibacterial proteins viz gloverin and lysozyme.

6. In order to identify the immune gene activation after infection by the fungus, *Beauveria* sp, larval integument samples from five bivoltine breeds were collected at 24h post infection. Total RNA was extracted from the integument samples. Five antifungal genes viz Amidase, Arylphorin, Glucose transporter, Gloverin and Lysozyme showed differential expression in semiquantitative expression analysis. Based on the real time PCR analysis, two divergent

races were selected for bioassay and correlation analysis between antifungal gene expression and fungal multiplication rate. The identified genes will be used for screening silkworm accession to use them as parents for synthesizing silkworm breeds with fungal tolerance.

7. In order to improve the resistance to BmNPV infection in *Bombyx mori,* a new disease management approach 'transkingdom RNA interference' has been initiated. The sequencing information of six NPV genes *ie 1, lef1, lef3 ,p74, p35* and *p143* involved in BmNPV infection has been obtained through cloning and BLAST analysis. Further these genes have been cloned into L4440 vector and dsRNA production for two genes *ie1* and *Lef1* has been initiated.

8. To identify molecular markers associated with the silk filament characters and to utilize these molecular markers for improving the multivoltine races, functional gene marker-association analysis is initiated. Several genes that are associated with the filament characters have been identified from various transcriptome analyses. Expression of twelve genes was analyzed in different regions of silk glands of selected multivoltine accessions at different time points during the phago period. Out of 12 genes, three genes associated with serine- glycine metabolism pathway, fibroin-H and fibroin–L genes showed variation in the gene expression.

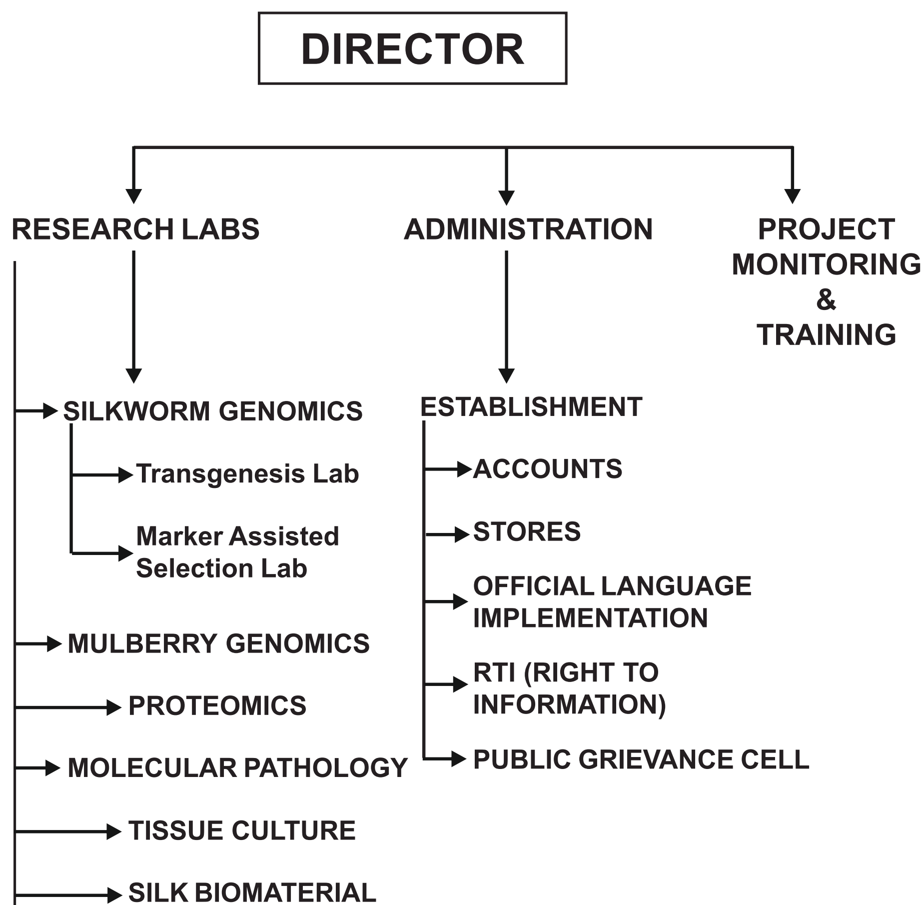
9. For developing Densovirus resistant- double hybrid based on the presence of DNV- resistant and susceptible markers, screening for parents of foundation cross are initiated. BBE266 and CSR6 are identified as parents to develop double hybrids and presently BBE266 is used as donor parent for developing back cross with CSR2 and CSR27. Further 30 bivoltine breeds carrying densovirus-2 resistance gene either in homozygous or heterozygous condition were also identified.

10. VgR gene is identified as a functional marker to identify accessions producing higher quality eggs. Breeds with high VgR transcript levels are shown to be producing larger number of eggs revealing positive correlation indicates its use in screening promising silkworm races for the selection of high fecundity.

11. The full length genome of Indian isolate of BmBDV (DNV) has been sequenced (NCBI accession number KX760110 and KX779526). Phylogenetic and sequence analysis have revealed close similarity of the Indian isolate of BmBDV with the Japanese isolate of BmBDV.

12. Prevalence of densovirus resistance gene (*nsd-2*) was screened in 20 bivoltine (BV) and 12 multivoltine (MV) races out of which APSHT–P5 (BV) and MV-76 & MV-77 (MV) revealed the presence of *nsd-2* resistant gene in homozygous condition in all the individuals tested indicating the availability of Indian silkworm germplasm resources with probable resistance to Indian isolate of BmBDV.

ORGANIZATION CHART



LIST OF RESEARCH PROJECTS

ONGOING RESEARCH PROJECTS

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)
2. **AIT 3538:** Development of Fibroin Fusion Silk with Antioxidant and Antibacterial Properties. (Jun 2015-May 2019)
3. **AIT 3540:** Development of Transgenic Silkworms for the Over-expression of Disease-Resistant Genes for Enhanced Immunity. **(In collaboration with IISc Bengaluru) (**Aug 2015-July 2018)
4. **CFC 7064:** Sericin for cosmetic applications **(In collaboration with CSTRI and M/s Unilever Industries, Bengaluru) (**May 2015-April 2017**)**
5. **ARP 3522 :** Isolation, cloning, and characterization of antibacterial proteins from silkworm, *Bombyx mori* **(In Collaboration from CSRTI, Berhampore)** (May 2015-April 2018)
6. **AIT 5872:** Whole Genome Sequencing and functional genomics of Golden Silk Moth *Antheraea assamensis* **(In Collaboration with IISc, Bengaluru, IIT, Guwahati, CDFD, Hyderabad, CMERTI, Ladoigarh)** (Oct 2015-Sep 2018)
7. **AIT 3582:** Development of densovirus resistant productive bivoltine silkworm breeds through marker assisted selection **(In collaboration with CSR&TI, Mysore)** (Sep 2016-Aug 2018)
8. **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)
9. **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, Bengaluru)** (Sep 2016-Aug 2019)
10. **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) (February 2017 – 2020)

11. **ARP 3606:** Development of diagnostic tool for early detection of baculovirus causing tiger band disease in *Antheraea proylei* **(In collaboration with CTRTI-RTRS, Imphal, Manipur)** (February 2017 – 2020)

CONCLUDED RESEARCH PROJECTS

1. **ARP 3513 (DST-JSPS Indo- Japan Collaborative Project):** Molecular characterization of Indian isolate(s) of Densovirus (DNV) and viral resistance gene in the host, silkworm *Bombyx mori* (June 2014-May 2016)

2. **AIT 3544 (CSB):** Validation of Vitellogenin Receptor (VgR) Gene Expression Levels as Molecular Indicator for Fecundity and Fertility in Silkworm Races. **(In Collaboration with CSGRC Hosur)** (July 2015- June 2016**)**

3. **AIT 3468 (DBT):** Development of RNA interference (RNAi) based nuclearpolyhedrosis virus resistance transgenic silk moth. (Jointly with CDFD, Hyderabad, APSSRDI, Hindupur) (Under Center of excellence on Genetics and Genomics of Silk moth to CDFD Hyderabad-Phase II) (Sept.2011- March 2017)

4. **AIT 3494 (DBT)**: Host-parasite interaction: Transcriptome responses to parasitism in the silkworm *Bombyx mori* (Jan.13- July.16).

PROGRESS OF ONGOING RESEARCH PROJECTS

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

Duration: Oct. 2014 - Sep.2017

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

Beauvaria bassiana causes white muscardine in *Bombyx mori* resulting in substantial crop loss especially during humid, rainy and winter seasons. Reports reveal upregulation and down regulation of several antifungal genes on *B.bassiana* infection in *B.mori*. Some of these genes have been validated through Real time PCR. Hence, a project was taken up in collaboration with CSGRC Hosur to investigate the differential expressions of the antifungal genes and its correlation with fungal proliferation among different *B.mori* breeds. The resultant outcome could indicate breed- specific response of the antifungal genes and their probable feasibility as markers for susceptibility / resistance to muscardine disease in *B.mori* breeds.

A comparative expression analysis of 14 antifungal genes *viz.* Lysozyme, LIM protein, Transferrin, Arylphorin, Glucose transporter, Peptidoglycan precursor, Ecdysone induced protein, Chemosensory protein, Bm8 interacting protein, Troponin C, Amidase, Gloverin, Neutral Lipase and Vacuolar ATP synthase was carried out in *B.bassiana* infected and control samples of 15 Multivoltine and 14 bivoltine silkworm breeds.

Differential expression analysis of antifungal genes in multivoltine races

From the semi quantitative expression analysis of 14 antifungal genes we shortlisted five antifungal genes *viz. Neutral lipase, Amidase, Glucose transporter, Arylphorin,* and *Gloverin* based on their differential gene expression in the multivoltine (MV) races . The MV races were also analyzed for the *B.bassiana* gene for determining the fungal multiplication and the results revealed that APDR15 (highly productive Breed) and Nistari (Popular Breed) showed lower fungal multiplication in contrast with *C. nichi* and MH1 (highly productive Breed) that showed higher fungal multiplication as indicated by increased copy number of fungal multiplication gene (Fig 1). Real time PCR analysis was carried out for the 14 multivotine races using five shortlisted antifungal genes.

APDR15 and Nistari revealed higher expression of antifungal genes in infected samples and *C. nichi* and MH1 revealed decrease in antifungal gene expression after infection indicating tolerance/susceptibility to fungal multiplication respectively (Fig 2**.** Therefore APDR15 and MH1 were short-listed among the highly productive breeds and *C. nichi* and *Nistari* from popular breed for further studies at different time points.

Differential expression analysis of antifungal genes in bivoltine races

From the semi quantitative expression analysis of 14 antifungal genes five antifungal genes *viz. Glucose transporter, Amidase, Gloverin, Arylphorin and Lysozyme* were short listed based on their differential gene expression in the 14 bivoltine races . The BV races were analysed for the fungal multiplication using specific *B.bassiana* fungal multiplication primers and the results revealed that the BV races, A and J2(P) showed lower fungal multiplication and CSR 27 and CSR 4 revealed higher fungal multiplication indicating susceptibility/tolerance to fungal infection.(Fig 3). The breed A and J2(p) among the autumn top performing breeds, breeds APS5 and APS4 among robust breeds, CSR27 and CSR 4 among popular breeds, and Barupat among low performers revealed higher expressions of the genes in infected compared to control. Comparison of the genes tested revealed that, the breeds J2(P) and A revealed higher expressions of the antifungal genes and CSR 4 and CSR 27 revealed lower expressions of the antifungal genes (Fig 4**.**

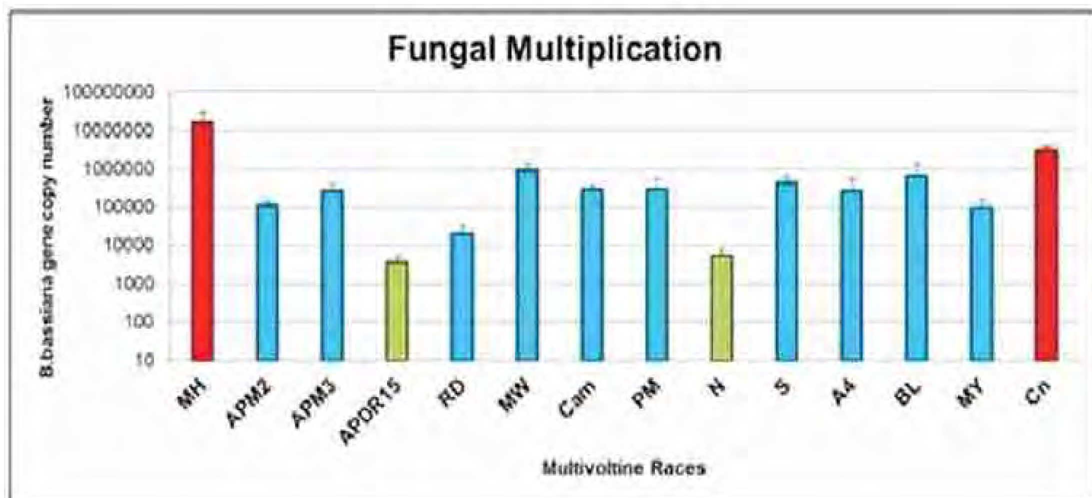


Figure 1: *Beauvaria bassiana* gene copy number (Fungal Multiplication) in multivoltine races

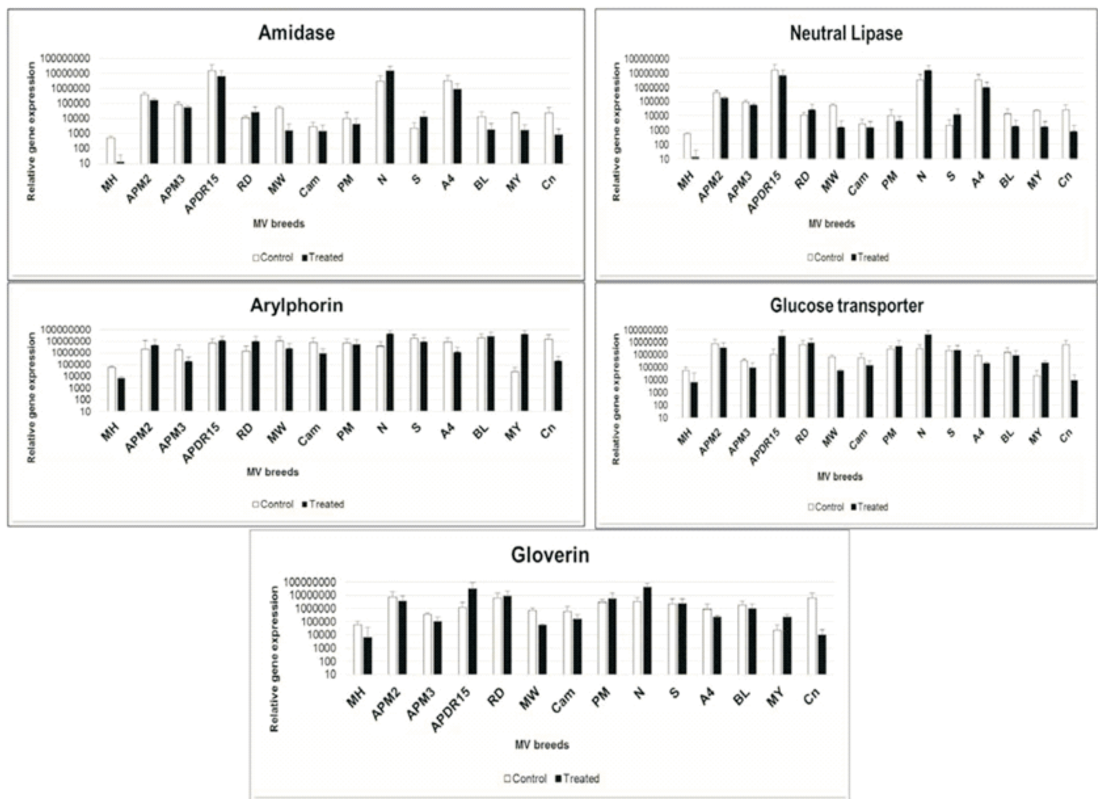


Figure 2: Relative gene expression of antifungal genes Amidase, Neutral Lipase, Arylphorin, Glucose transporter and Gloverin in control (without infection) and Treated (with Infection) multivoltine races**.**

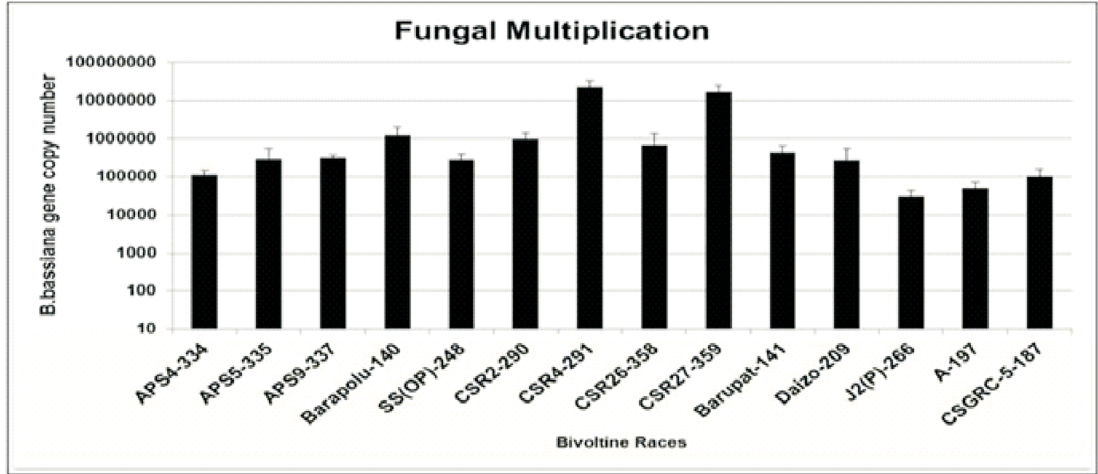


Figure 3: *Beauvaria bassiana* gene copy number (Fungal Multiplication) in bivoltine races

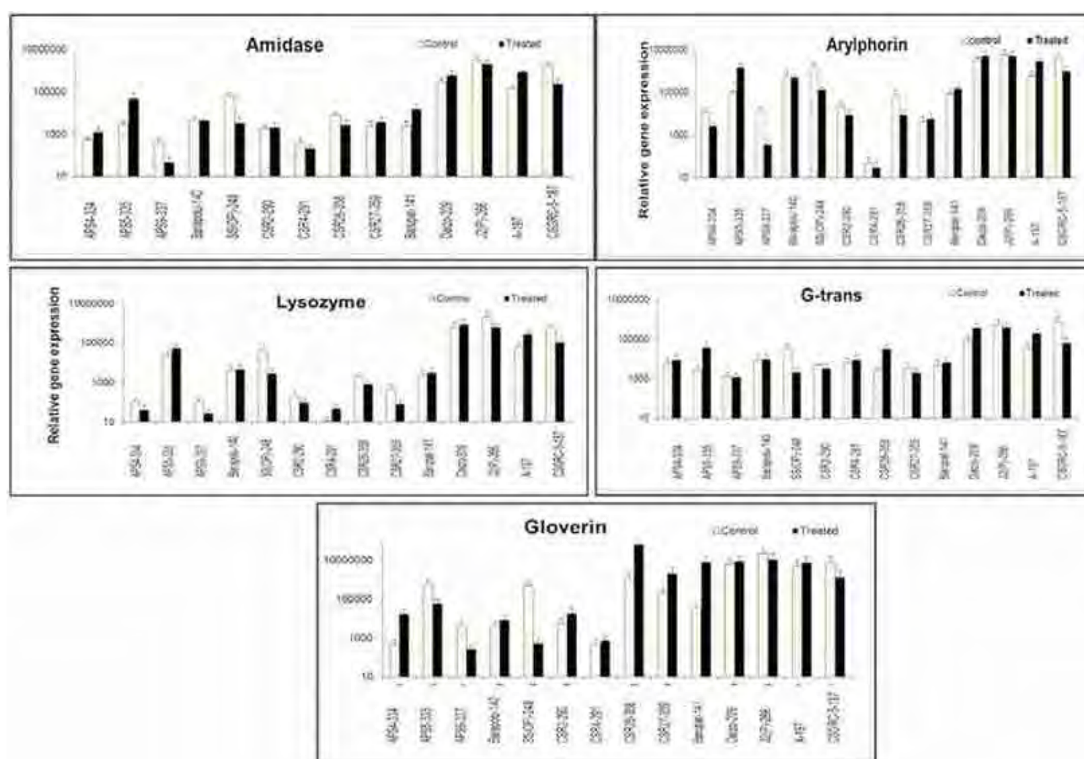


Figure 4: Relative gene expression of antifungal genes Amidase, Arylphorin, Lysozyme, Glucose transporter and Gloverin in control (without infection) and *B. bassiana*- infected bivoltine races**.**

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

Duration: July 2015 – June 2019

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

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

Silk fibroin, Cecropin B and fibroin-Cecropin fusion proteins were expressed in the yeast *Pichia pastoris* expression system and in cell-free system. The recombinant proteins were detected by Western blotting and purified by Immobilized Metal Affinity Chromatography (Fig 5). The purified protein will be tested for wound healing and

antibacterial assays. Vector constructs are underway to express the same fusion protein in transgenic silkworms using the transposon vector *piggy Bac* and fibroin gene expression system.

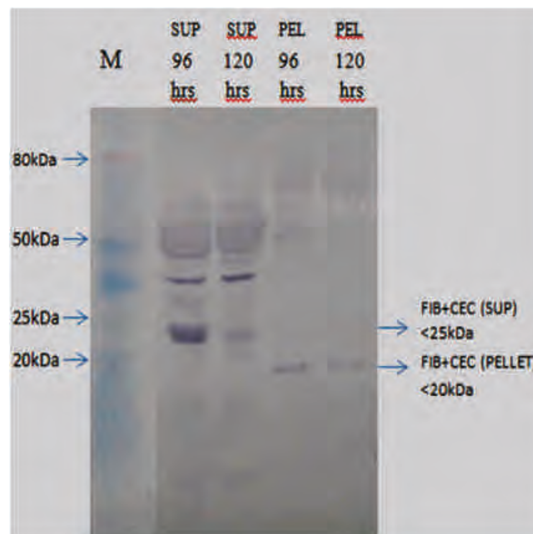


Figure 5: Western blot with anti - histidine antibody showing the expression of fusion protein at 96 and 120 hrs.

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

Duration : June 2015 - July 2018

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

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

Relish, Cecropin B and *Drosomycin* genes and their promoters were constructed in *piggyBac* plasmids. To test the promoter activity of CecA, CecB and Drosmycin, GFP wer cloned downstream to these promoters. These were injected/electroporated in to pre-blastoderm silkworm eggs. Observations are underway.

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

Duration : Oct 2015 - Sep 2018

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

Muga silkworms of wild and commercial populations were collected and reared at CMERTI, Jorhat and presently under multiplication. Among the individuals of *A. assamensis*, homozygosity test was conducted using *Antheraea assamensis* specific microsatellite primers (*Aa*Sat) on agarose gel. Homozygosity analysis in commercial population of muga silkworm was analyzed in 16 individuals after genomic DNA isolation. Seven SSR primers were used to asses homozygosity level in 16 individuals revealed that the commercial population is highly homozygous confirming homozygosity in populations of the muga silkworm found earlier (Fig. 6).

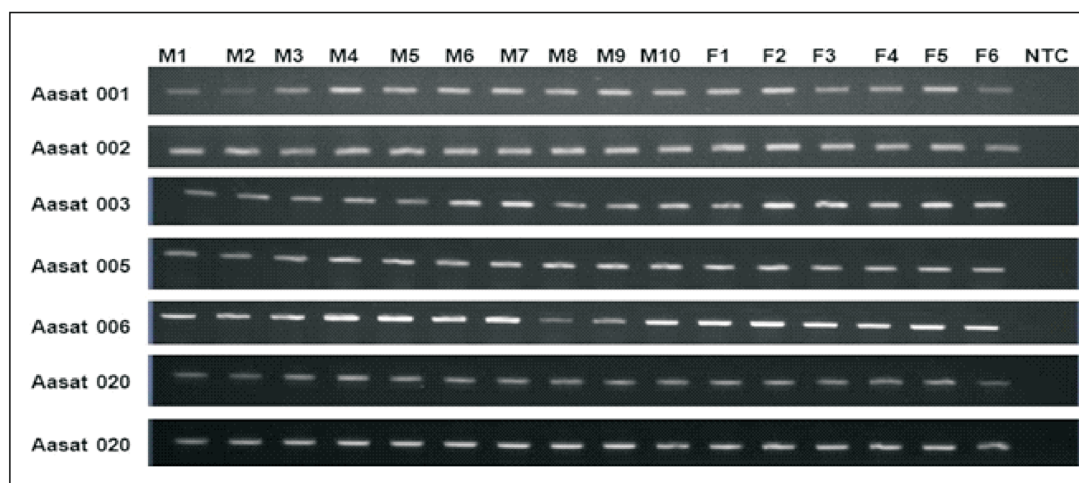


Figure 6: Homozygosity test conducted for muga silkworm, *Antheraea assamensis* provided by CMERTI, Jorhat using AaSat microsatellite primers. M- male; F- female; NTC- Non-template control

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

Reporting period: (Sept. 2016 to Mar. 2017)

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

Viral diseases account for majority of the total cocoon loss due to pathogens. *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV) and *B.mori* densovirus (*Bm*DNV) are the two major prevalent viruses. Flacherie disease is one of the widespread and severe diseases in silkworms, causing up to 20% crop loss. *Bm*DNV is one of the major pathogen causing flacherie disease in silkworm. Major symptoms associated with *Bm*DNV infections are flaccid larval body and diarrhoea and turn to dark brown and finally die. It is essential to develop an appropriate disease management strategy to control the disease. Other than disinfection, development of disease resistant breeds is a prominent approach to control the disease outbreak. In this back drop, 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 **(**Ito K, et al. (2008) Proc Natl Acad Sci USA **105**:7523–7527**)**

This project aims to develop DNV-2 resistant productive bivoltine silkworm breeds/ hybrids using two approaches. First approach is introgressing *nsd-2* resistance gene from the resistant parent to CSR breeds by marker- assisted backcross method. Other approach is to identify DNV-2 resistant productive bivoltine breeds from the breeding stock and utilize them for hybrid development.

During the period under report, 45 productive silkworm breeds collected from CSR&TI, Mysore and from CSGRC, Hosur were screened for the presence of DNV-2 resistant allele (is it correct terminology) through PCR. Genomic DNA was isolated from the moths and two sets of primers were used to identify resistant and susceptible allele of *nsd-2* gene (Fig 7**).** We have identified 27 breeds carrying DNV-2 resistant allele either in homozygous or in heterozygous condition (Table 1**).** The identified breeds carrying DNV-2 resistant allele can be used to develop DNV-2 resistant productive silkworm breeds.

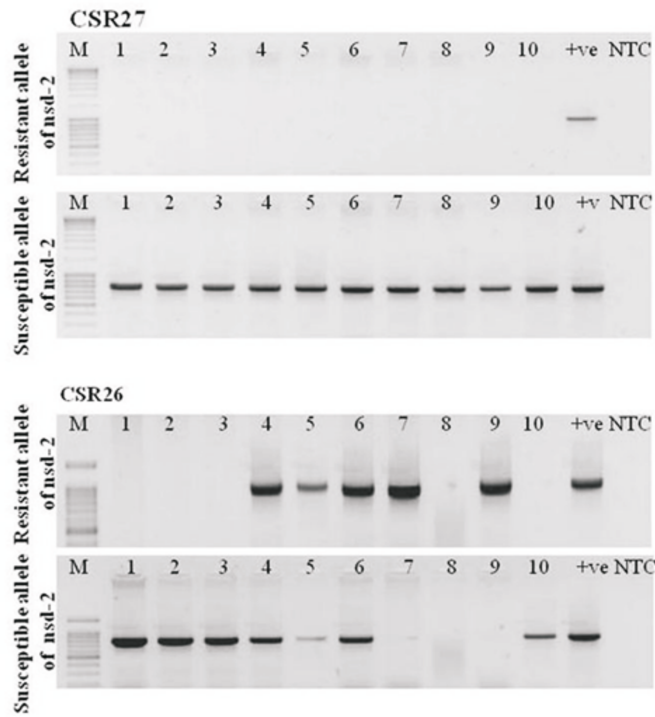


Figure 7: Screening for DNV-2 resistant allele in CSR27 and CSR26 through PCR: Two sets of primers were used specifically to identify resistant and susceptible allele of nsd-2.

Table 1: List of silkworm breeds screened for presence or absence of DNV-2 resistant allele

Sl. No.	Bivoltine Breed	Presence/Absence of nsd-2 resistant allele	Sl. No.	Bivoltine Breed	Presence/Absence of nsd-2 resistant allele
1	CSR2	Absent	24	A110	Present
2	CSR4	Absent	25	APSHT05	Present
3	CSR17	Absent	26	PAM117	Present
4	CSR27	Absent	27	21D	Present
5	S5	Absent	28	BBE-0263	Absent
6	S8	Absent	29	BBE-0264	Absent
7	N1	Absent	30	BBE-270	Absent
8	N2	Absent	31	BBE-0332	Absent
9	N3	Absent	32	BBE-0177	Present
10	N4	Absent	33	BBE-0178	Present
11	D7	Absent	34	BBE-0179	Present
12	O-Plain	Absent	35	BBE-0186	Present
13	APS9D	Absent	36	BBE-0188	Present
14	EC1	Absent	37	BBE-0197	Present
15	CSR6	Present	38	BBE-0198	Present
16	CSR26	Present	39	BBE-0232	Present
17	CSR51	Present	40	BBE-0252	Present
18	N5	Present	41	BBE-0262	Present
19	N6	Present	42	BBE-0266	Present
20	N7	Present	43	BBE-0267	Present
21	N11	Present	44	BBE-0364	Present
22	S1	Present	45	BBE-0370	Present
23	Chi-o	Present			

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

Duration : Sep 2016 - Aug 2019
Reporting period: (Sept. 2016 to Mar. 2017)

K. S. Tulsi Naik and K. M. Ponnuvel
M. V. Rajam [UDSC, New Delhi]
Mrs. Shambhavi P Hungund [JRF]

Bombyx mori L. (Lepidoptera: Bombycidae) nucleo polyhedrosis virus (BmNPV) is a highly pathogenic virus encountered in the sericultural industry, often causing severe damage which hampers silk cocoon production in tropical countries. Effective management of the virus has been a challenge because of its sturdy nature and the lack of control strategies. RNA mediated silencing technology has become present day's tool of choice for induction of virus resistance in most of the higher animal systems. In this context, a new approach, known as transkingdom RNA interference (tkRNAi), was discovered where the *E. coli* are engineered to transcribe short RNA (shRNA) from a plasmid known as transkingdom RNA interference plasmid (TRIP). This method utilizes attenuated, non-pathogenic bacteria that are safe, effective, and inexpensive vectors for delivering RNAi to target cells. Bacteria-based RNAi is technically suitable for production of large quantities of dsRNA, and therefore opens interesting perspectives for mass screening of novel gene targets and development of environmentally-safe sericulture applications.

In the above context two genes IE-1, a transregulator that is responsible for activation of a number of late expression factors (encoded by *lef* genes), LEF-1, a DNA primase of the replication complex (Mikhailov and Rohrmann 2002) are the most important factors in viral DNA replication. The gene sequence was analyzed for the presence of potential siRNA using Whitehead SiRNA software analysis tool and found that there are around 7 potential SiRNA in the selected sequences

Cloning of IE1 and LEF 1genes into L4440Vector

The IE1 and LEF 1 genes were PCR amplified from BmNPV genomic DNA and cloned into pJet vector. **(Fig 8).** After confirmation of the sequences, both genes were subcloned into Bacterial L4440 vector and clones were screened by restriction analysis

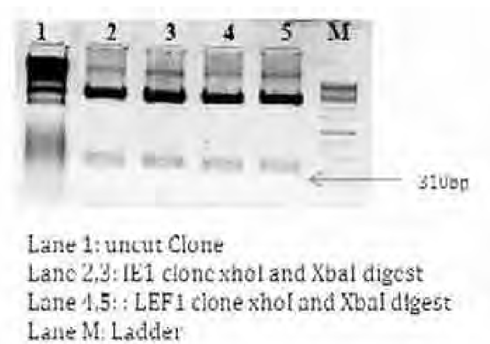
for confirming the insert of 300bp for both the genes (Fig 9). The confirmed clones were transformed into *E.coli* HT115 host strain that is RNase III deficient for expressing dsRNA against both the genes.

Expression analysis of Ie1 and Lef1 dsRNA in bacteria

E. coli HT115 strain harbouring plasmid L4440-Ie1 as well as L4440 Lef 1and empty vector L4440 was grown overnight in an incubator shaker at 37^0C and 250 rpm. One per cent of inoculum was transferred to 50 mL LB broth and grown at 37^0C and 250 rpm. At OD 600 ¼ 0.6, the cultures were induced with 0.25 mM IPTG and grown for another 4 hrs. The cultures were then harvested by centrifugation at 8000 rpm for 5 min at 25^0C, followed by RNA isolation using RNA isoplus (Takara Bio) as per the manufacturer's instructions. The RNA samples were quantified using Nanodrop™ and used for cDNA preparation using Prime script cDNA synthesis kit (Takara Bio). The resulting cDNAs were then PCR amplified using gene-specific primers and checked on 1.5% agarose gel.

E.coli HT115 strain lacking the RNaseIII activity was used to express the dsRNA against IE1 and LEF 1genes. The expression of dsRNA was checked by semi-quantitative RT-PCR, which showed the amplification of desired sequence with specific primers (Fig. 10). IPTG induced T7promoter was hence found to transcribe the desired sequence in bacteria, which was then used to feed the insect larvae for studying gene silencing. Further studies include standardization of feeding bioassay experiments and dosage of dsRNA that is to be fed to study the effect of dsRNA on the targeted genes is underway.

Figure 8: Restriction analysis of pJET1.2 clones to release the 310bp insert Lef 1 and Ie1



Figue 9: Restriction analysis of L4440 clones to release the 310bp insert Lef 1 and Ie1

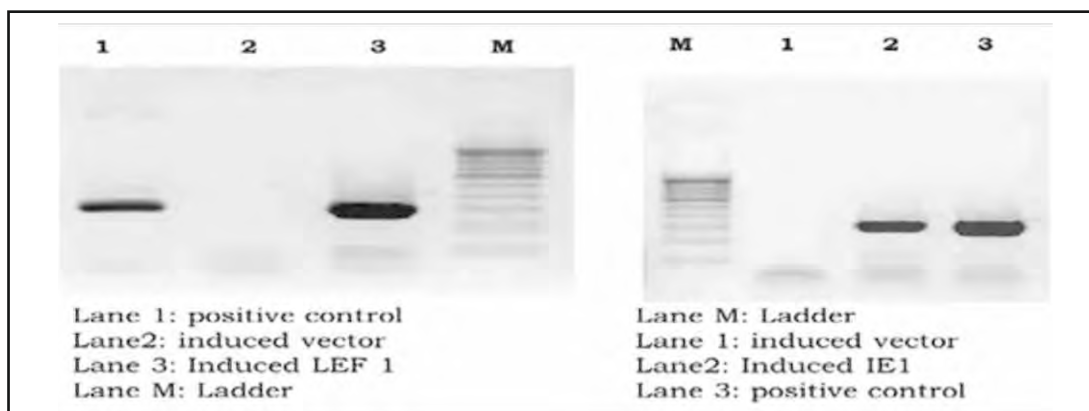


Figure 10: Expression analysis of genes sub cloned into L4440 vector by semi quantitative PCR.

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

Reporting period: (Sept. 2016 to Mar. 2017)

K. S. Tulsi Naik and A.R. Pradeep

G. Hariraj [CSTRI, Bangalore], L. Kusuma [CSR&TI, Mysore]

Mrs. Shambhavi P Hungund [JRF]

The silkworm, *Bombyx mori*, is an agriculturally important economic insect that has been used for silk production. Though India is today the second largest producer of silk in the world, the twin problems of low productivity and poor fibre quality continue to impair an increase in production. Though the bivoltine breeds/hybrids are known for their productive merit, absence of genetic plasticity to buffer against adverse conditions prevailed in the field acts as a constraint to exploit the full economic potential of these new hybrids and these breeds/hybrids continue to suffer badly in adverse conditions prevailed in the field.

Multivoltine silkworms are best suited for the tropical climates of India. Performances of multivoltine silkworms are better in terms of their robustness, adaptability to the changes in the environment and most importantly the disease resistance compared to bivoltine. But as far as the post cocoon parameters are concerned, the performance

of multivoltine is low compared to bivoltines in terms of shell weight, cocoon shell percent, filament length, denier, reelability, non breakable filament, raw silk percentage and neatness. More than 90% of silk produced in India is either from multivoltine or (multi) x bivoltine (bi) hybrids. Silk produced in India, particularly of multivoltine silkworms, suffers from variation in the denier which breaks during winding and weaving and thus is not preferred as a warp in the power loom. If Indian silk can be better with respective denier uniformity and strength, it can be wound without breaks on automatic reeling machines and the thread can be used in power looms as it has a better lustre and dye affinity.

Importance of the study

The situation analysis and future strategy for multivoltine silkworm breeding suggest for the improvement of the multivoltine in terms of their economic characters. In this context, identification of molecular markers for fibre quality and molecular characterization of multivoltine races utilizing the markers to improve the multivoltine breeds by marker assisted selection are important. The comparison of the quantitative filament characters in bivoltines and multivoltine silkworms are listed.

Important parameters for grading of silk yarn

(Filament characters)

1. Reelability,
2. Neatness,
3. filament size and length,
4. Raw silk percent,
5. Boil off loss ratio
6. Lousiness,
7. Winding breaks,
8. Eveness,
9. Tenacity and
10. Cohesion

The silk gland is the only organ where silk proteins are synthesized and secreted in the silkworm (Fig 11). Silk proteins are stored in the lumen of the silk gland for around eight days during the fifth instar. Determination of dynamic changes in different regions of silk glands is helpful to clarify the secretion mechanism of silk proteins. The recent progress in the construction of molecular genetic maps, BAC libraries and a variety of molecular marker assays will further widen the scope of genetic analysis of this organism.

In the above context this project was proposed to identify molecular markers for the filament characters, that includes filament size, neatness of the filament, reelability, raw silk percentage in multivoltine silkworms. Since the filament characters are controlled by poly genes identification of combination of multiple markers/genes responsible for better filament characters is the objective of this project. Based on the passport data from silkworm catalogue published by CSGRC Hosur, five each of low performing and high performing multivoltine races (with respect to filament characters) were identified. These races were collected from the Silkworm germplasm and reared under prevailing conditions. Anterior, middle and posterior silk glands were dissected out from seven multivoltine races of fifth instar on day 1,3,and 6 of phago period and isolated genomic DNA and total RNA. cDNA was synthesized to analyze the gene expression of 12 genes that are associated with filament characters. Out of 12 genes, three genes, fibroin light chain region, fibroin heavy chain region, and BGIBMGA009925 (Fig 12) that is involved in serine glycine metabolism pathway showed variations in gene expression. Further experiments are in progress.

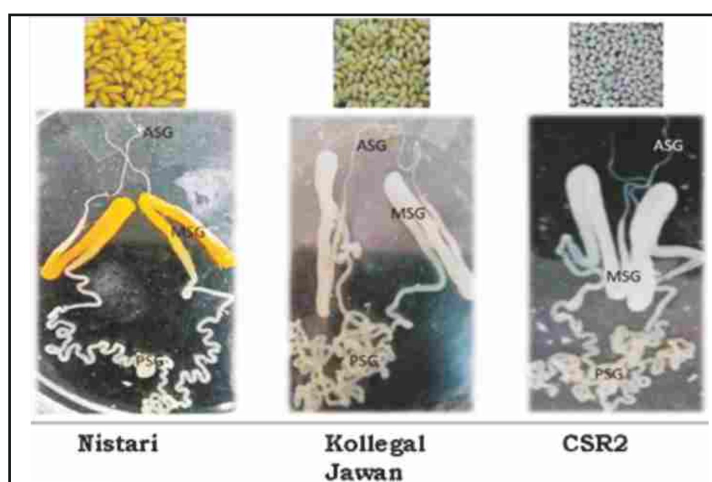


Figure 11 : Silk glands dissected from different silkworm races

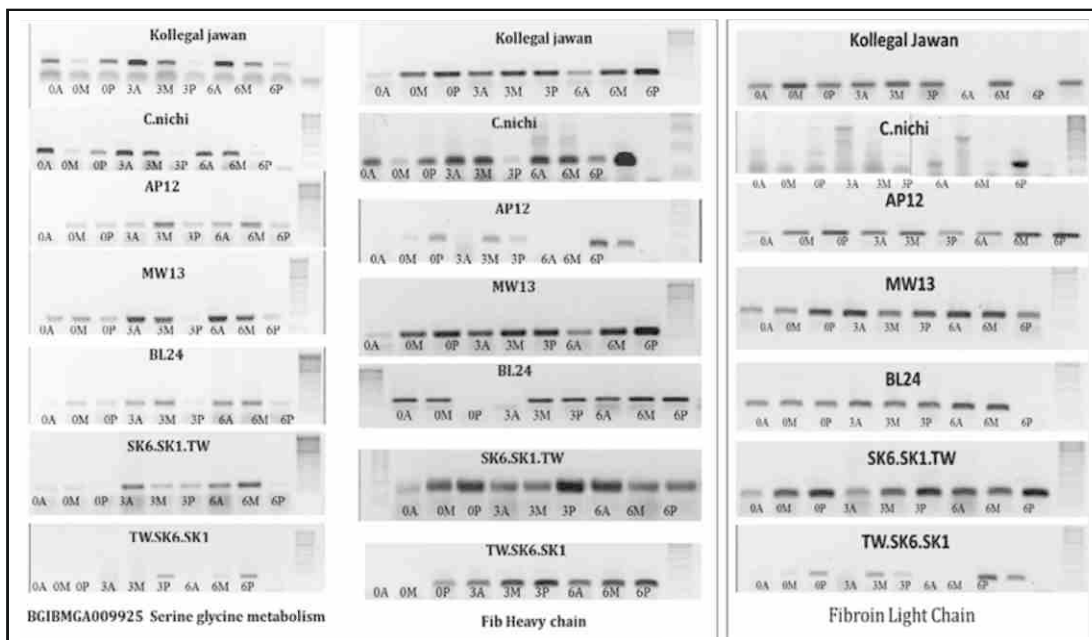


Figure 12: Differential expression analysis of three genes associated with filament characters in selected multivoltine races

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.

(Network project of SBRL, CSRTI- Mysore, CSRTI- Berhampore and CSRTI-Pampore, funded by Department of Biotechnology, Govt of India, New Delhi)

Duration : Feb 2017 – Jan 2020

Report period: February – March 2017

Project co-ordinator: Dr. V. Sivaprasad, Director, CSR&TI, Mysore

Manthira Moorthy, CSRTI, Mysore and B. Mohan, SSBS, Coonoor ,
Gopal Chandra Das, and S. Chandrakanth, CSR&TI, Berhampore,
Mukesh Tayal, Regional Sericultural Research Station, Miransahib, Pampore,
Mohammed Aslam, CSR&TI, Pampore, J&K,
Pankaj Tiwari, Regional Sericultural Research Station, Dehradun, Uttarkhand,
A. R. Pradeep and K.M.Ponnuvel, SBRL, Bangalore

Objectives (SBRL)

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

CSRTIs of Mysore, Berhampore and Pampore

- ✯ To evaluate the evolved bivoltine lines in various agro climatic conditions and select lines for their suitability at different regions
- ✯ Preparation of Dfls of PM / Nistari (or other promising multivoltine of the area) X MASN and CSR4 x MASN crosses through NSSO, Bangalore and distributed to Sericulture Farmers of area under CSRTI, Mysore

Research progress

The project is initiated in February 2017. First batch of DFLs of MAS-N lines 4, 6 and 7 were send to CSRTI Berhampore and Pampore for stock development. At CSR&TI, Mysore, rearing will be initiated at the onset of favorable environmental conditions after peak of summer followed by multiplication at SSBS, Coonoor.

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

Duration : Feb 2017 – Jan 2020

Report period: February – March 2017

K.M Ponnuvel (SBRL, Bangalore), Sinam Subhrani Devi (RTRS Imphal), Ms. Shruthi (JRF) & Ms. Aarthi (Proj Asst.)

Objectives

1. To characterize the baculovirus pathogen causing tiger band disease in oak tasar silkworm, Antheraea proylei
2. To study the pathogenesis, source and mode of transmission of the viral pathogen
3. To develop DNA based diagnostic tools for early detection of baculovirus causing tiger band disease
4. Validation of developed diagnostic tools in Oak tasar Grainages and egg production centers

Progress:

The project is initiated in February 2017. Collection of infected *A. proyeli* larvae is underway at RTRS, Imphal.

COLLABORATIVE PROJECTS OF OTHER INSTITUTES

CFC 7064 : Sericin for cosmetic applications (In Collaboration with CSTRI, Bengaluru and M/s Unilever Industries, Bengaluru)

Duration: May 2015 – April 2017

G. Ravikumar [SBRL, Bangalore]
Mrs Chitra, M, JRF [SBRL]
Mrs. Dyna Susan Thomas, Research Fellow [SBRL]
M.A. Joseph, [CSTRI, Bangalore]
Jyoti Tiwary, [M/s Unilever Industries, Bangalore]

Aim: To characterize Sericin proteins from cocoons and silk gland.

Progress

Sericin extracted from silk glands of CSR2 larva and Sericin powder made from cocoons were resolved by SDS-PAGE. The cocoon sericin appeared as smears of different molecular weights whereas discrete banding patterns were observed fromprotein extracted from the silk glands. Further analysis is under progress at Unilever Industries.

ARP 3522 : Isolation, cloning, and characterization of antibacterial proteins from silkworm, *Bombyx mori* (In Collaboration with CSRTI, Berhampore)

Duration: May 2015 – April 2018

Kanika Trivedy, Rahul and Zakir Hussain [CSRTI, Berhampore]
Mr. Gourab Roy, [JRF] and G. Ravikumar [SBRL]

Objectives: To clone and characterize antibacterial proteins from silkworm, *Bombyx mori*

Analysis of silkworm haemolymph proteins collected after *Staphylococcus vitulinus* bacterial infection on cross breed of *B. mori,* M.com4 x B.com4 by SDS-PAGE analysis and mass Spectrometry showed presence of significant number of peptides of antibacterial proteins viz gloverin and lysozyme. Proteins up-regulated by bacterial

infections were analysed by MALDI/TOF. Five proteins were short-listed based on their homology to antibacterial proteins and sequence features. Primers were designed from these genes encoding these proteins, RNA isolated from fat bodies, first strand cDNAs were synthesized and PCR amplifications were done. The PCR products were cloned and clones were sent for sequencing.

CONCLUDED RESEARCH PROJECTS

DST-JSPS Indo-Japan Collaborative project

ARP 3513 : Molecular characterization of Indian isolate(s) of Densovirus (DNV) and viral resistance gene in the host, silkworm *Bombyx mori*

Ponnuvel K. M.[1], Tania Gupta[1], V. Sivaprasad[1], Keiko Kadono-Okuda[2], Yumiko Nakajima[3], Katsuhiko Ito[4]

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The complete nucleotide sequence of the Indian isolate of BmBDV has been determined and submitted to NCBI. The sequence data was assembled into two contiguous sequences named VD1 (6542 nts) and VD2 (6023 nts). The DNA polymerase gene located on the VD1 fragment was cloned and sequenced first followed by the cloning and sequencing of the subsequent regions of the VD1 fragment. The Genbank accession numbers assigned for VD1and VD2 DNA segments are KX760110 and KX779526, respectively, while the Genbank accession number assigned for DNA polymerase gene of Indian isolate of BmBDV is KP886818. The base composition of VD1 was found to be 71.12% A+T, while, that of VD2 is 67.92% A+T. Both VD1 and VD2 DNA fragments were found to possess high A+T content. The complete linear genomic text map of the VD1 and VD2 DNA segments of the Indian isolate of BmBDV has been given in Online Resource 1 and 2, respectively. As per previous reports, the terminal palindromic sequences commonly present in other densoviruses were not found in the Indian isolate of BmBDV, thereby depicting the typical feature of the *Bidnaviridae* family. VD1 was found to have inverted terminal repeats (ITRs) of 224nts, while, VD2 had ITRs of 499nts.The 'panhandle structure' formation by the single

stranded viral DNA molecule can be suggested by the structural analysis of both VD1 and VD2 ITRs. The 5' terminal end sequence from 1-224 nts of VD1 was found to be complimentary with that of 6318-6542 nts of 3' end terminal sequence. The GC content of Indian isolate of BmBDV ITRs (VD1:42.41%; VD2:43.34%) was found to be much higher than that of the entire viral genome (VD1: 32.06%, VD2: 28.86%).

The putative ORFs present in the genome of Indian isolate of BmBDV were identified using the NCBI ORF finder software. Methionine-initiated codon (ATG) and stop codons (TAA, TAG and TGA) along with amino acids with a minimum of 100aa in length and showing minimal overlap were some of the criteria kept for searching ORFs in the Indian isolate of BmBDV. Six putative ORFs were identified in the genome of Indian isolate of BmBDV. The VD1 DNA segment of the Indian isolate of BmBDV was found to have four ORFs while the VD2 DNA segment comprised of two ORFs, similar to the Yamanashi and BmBDV-Z isolates. The VD1 segment of DNA was found to contain four ORFs (VD1 ORF1, VD1 ORF2, VD1 ORF3 and VD1 ORF4), while, VD2 had two ORFs (VD2 ORF1, VD2 ORF2). The orientation and the number of putative ORFs identified in the Indian isolate of BmBDV resembled that of the Japanese and the Zhenziang isolates which have also been reported to have six ORFs in total.

To have a better understanding of the genomic structure of the Indian isolate of BmBDV, the distribution of initiation and polyadenylation signals were also analyzed by using the NNPP algorithm. The genomic organization of the VD1 and VD2 DNA segments along with the promoter signals have been shown in (Fig: 13a &13b), respectively. The first putative TATA box (TATATAA), on VD1, was found to be located between nts 259 and 265. This putative promoter might control the transcription of VD1 ORF1 and VD1 ORF2. Another TATA box was found to be located between nt 1401 and 1407, which possibly controls the transcription of VD1 ORF3. Further, a third TATA box was found located on the minus strand between nt 6300 and 6306. The VD1 ORF4 transcription might possibly be controlled by this promoter. Polyadenylation signals (AATAAA) were also found located between nt 1406–1411 and nt 2911- 2916. This signal was also found located on the complementary strand at nt 2943–2938. In case of the VD2 DNA sequence, a putative TATA box was found between nt 644 and 650 which could possibly control the transcription of VD2 ORF1. Also, a polyadenylation signal was noted between nts 650-655. However, no promoter signal was detected for the VD2 ORF2 gene sequence.

Transcript analysis through q-PCR

The identification of 6 putative ORFs for the Indian isolate of BmBDV was confirmed through q-PCR and the expression level of each of the ORFs was analyzed as well as compared through quantification. All the putative ORFs showed significant transcript levels. However, VD2 ORF1 and ORF 2 showed the maximum transcript level. Among all the six ORFs, VD2 ORF 1 was found to exhibit the maximum transcript level while VD1 ORF4 had the least expression (Fig. 14).

Comparison of the Indian isolate of BmBDV with other reported isolates

The comparison of the genome length, ORF length and ITRs of the Indian isolate of BmBDV with that of the other reported BmBDV isolates has been shown in (Table 2-5). The VD1 DNA segment of the Indian and the Japanese isolates are of the same length i.e. 6542bp, while the Chinese and the Zhenjiang isolates are 1bp larger than the Indian isolate (6543bp). However, the VD2 DNA segment of all the four isolates vary in their lengths with the Japanese isolate having the maximum length of 6031bp, while, the Chinese isolate has the shortest length of 6022bp. The Indian BmBDV and BmBDV-Z differ in their VD2 lengths only by one nucleotide wherein the Indian isolate is 6023bp in length, while, BmBDV-Z has a length of 6024bp. Further, the ORF characterization revealed the Indian, Japanese and BmBDV-Z isolates to have a total of 6 ORFs; four on the VD1 segment and two on the VD2 DNA segment. However, the Chinese isolate has been reported to have seven ORFs; four on the VD1 segment and three on the VD2 DNA segment. A single nucleotide "A" deletion at position nt 1583 of VD2 ORF1 in the Chinese isolate resulted in a frameshift mutation that brought about a premature stop codon. Consequently, the Chinese isolate has three ORFs on VD2 *viz.,* VD2 ORF1-a and VD2 ORF1-b. However, no such mutation was observed in the other isolates. Further, the Indian isolate was individually compared with each of the reported isolates. Tables 3, 4 and 5 show the comparison of the Indian isolate of BmBDV with that of the Japanese, Chinese and Zhenjiang isolates, respectively. The comparison of the Indian isolate with that of the Japanese isolate revealed a total of 221 bp out of which 159 substitutions were found to occur in the coding regions of the six ORFs. Consequently, 58 amino acid changes were observed in all the 6 ORFs of the Indian isolate of BmBDV. Among the 159 substitutions in the coding regions, 101bp substitutions were silent. Similarly, the comparison between BmBDV Z and the Indian isolate revealed a total of 310 bp substitutions out of which 159 substitutions occurred in the 6 ORF sequences just like the Japanese isolate. However, the overall number

of substitution was higher in this case wherein the substitutions majorly occurred in the non-coding region of the sequence. The comparison between the Chinese and the Indian isolates of BmBDV revealed a total of 271 bp substitutions out of which 153 substitutions occurred in the coding regions. The 153 substitutions brought about only 46 amino acid changes in the ORF sequences thereby suggesting that 118 nt changes were silent. The ITR comparison of the Indian isolate with that of the other isolates revealed the VD2 DNA segment of the Indian isolate to possess a 26 bp nucleotide addition on the 5’ terminal end.

Phylogenetic analysis of the Indian isolate of BmBDV

The phylogenetic analysis of all the reported isolates of BmBDV sequences was carried out using Mega6 software for studying the homology between the VD1 and VD2 DNA segments of the isolates. The scale range for analysis of VD1 DNA segment was from 0.001 to 0.005 (Fig. 15), while, in case of VD2 DNA segment the scale range was from 0.002 to 0.012 (Fig. 16). The scale range clearly indicates the fact that both VD1 and VD2 DNA segments share a close homology without any significant differences. However, in spite of the close homology between the VD1 and VD2 DNA segments, VD1 has a higher conserved homology than the VD2 DNA segment as indicated by the scale range. Further, the phylogenetic tree constructed revealed two separate clusters wherein the first cluster was formed by the Indian and the Yamanashi isolates, while, the Chinese isolate and BmBDV-Z isolates formed the second cluster. Phylogenetic analysis of each of the ORFs was also carried out separately wherein each of the ORFs revealed a close homology, which indicated towards the fact that the ORF sequences have been highly conserved in all the reported isolates of BmBDV. However, among all the ORFs, VD1 ORF 2 showed the maximum conserved sequences, while, VD2 ORF 1 and 2 exhibited the maximum variability in sequences. Among the two DNA segments the VD1 ORFs seem to have been highly conserved.

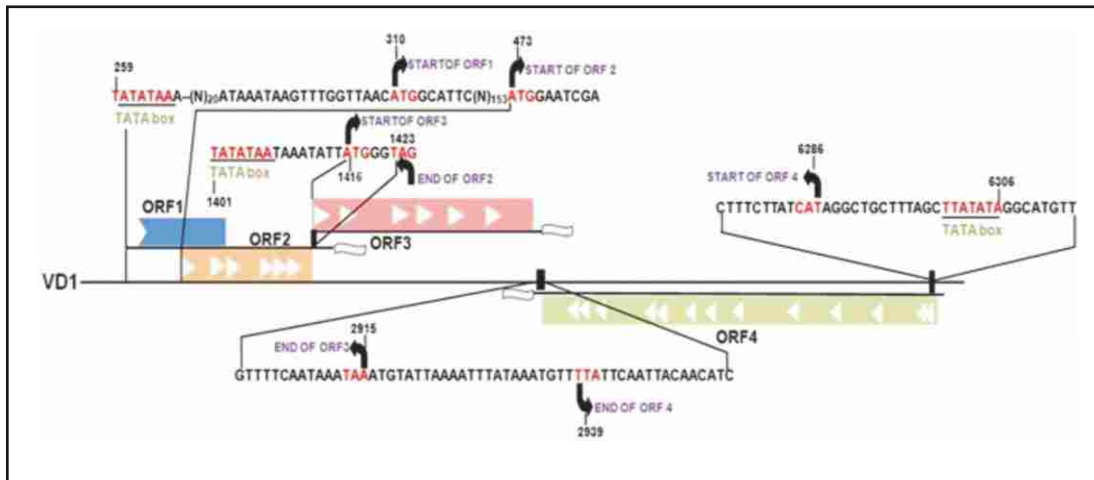


Figure 13a : The genome organization of VD1 DNA segment of Indian isolate of BmBDV. Horizontal line represents the VD1 DNA segment. The colored bars above the line represent the ORFs encoded by the positive strand and those below the line represent the ORFs encoded by the complimentary sequence. The TATA boxes indicate the functional promoters.

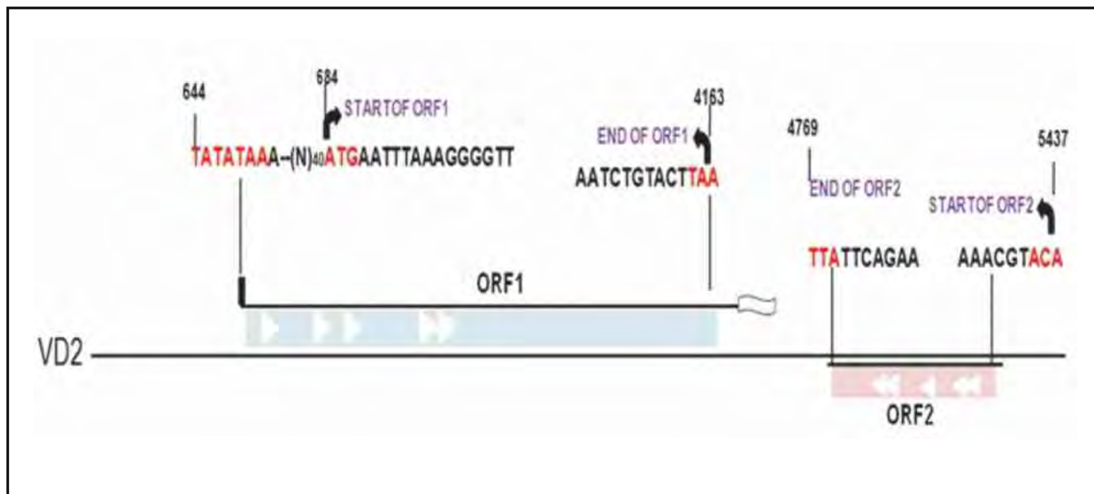


Figure 13b : The genome organization of VD2 DNA segment of Indian isolate of BmBDV. Horizontal line represents the VD2 DNA segment. The colored bars above the line represent the ORF encoded by the positive strand and the one below the line represents the ORF encoded by the complimentary sequence. The TATA boxes indicate the functional promoters.

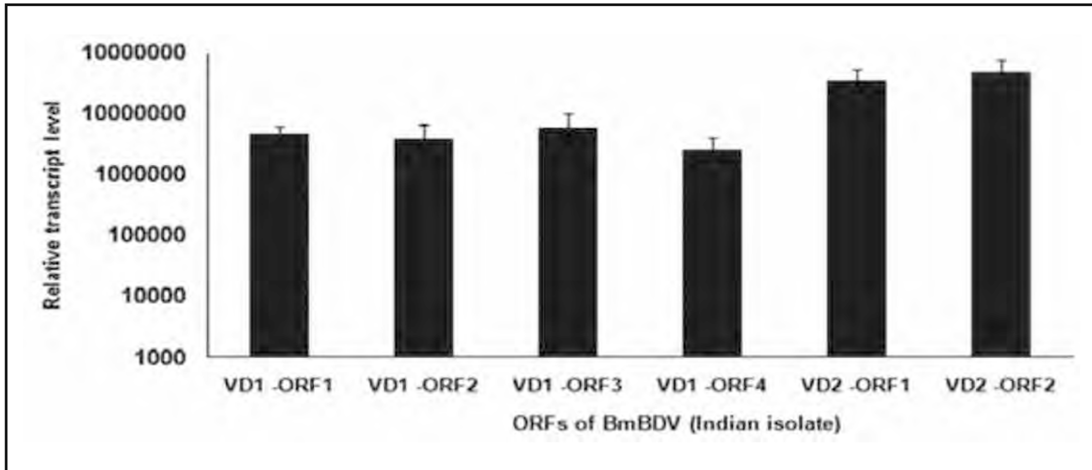


Figure 14: Differential level of viral ORFs expression in BmBDV (Indian isolate) infected silkworms.

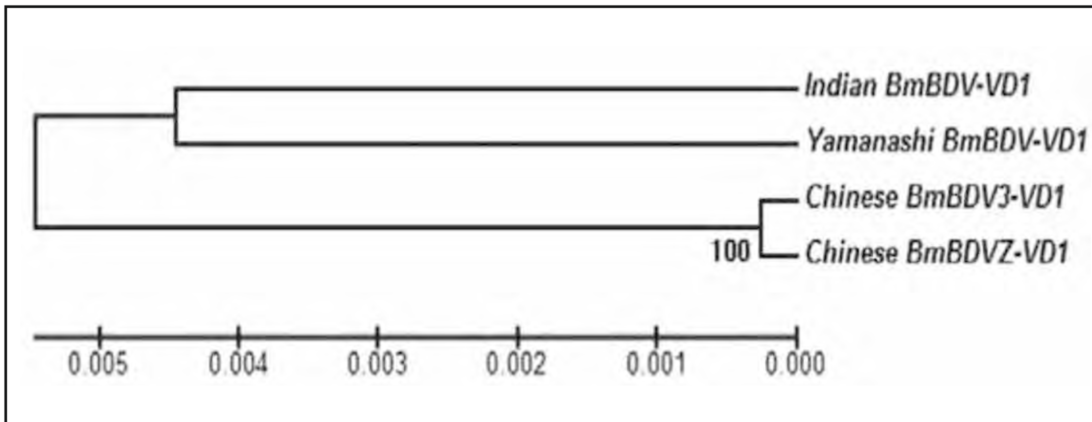


Figure 15 : Phylogenetic tree of VD1 DNA segment of Indian isolate of BmBDV.

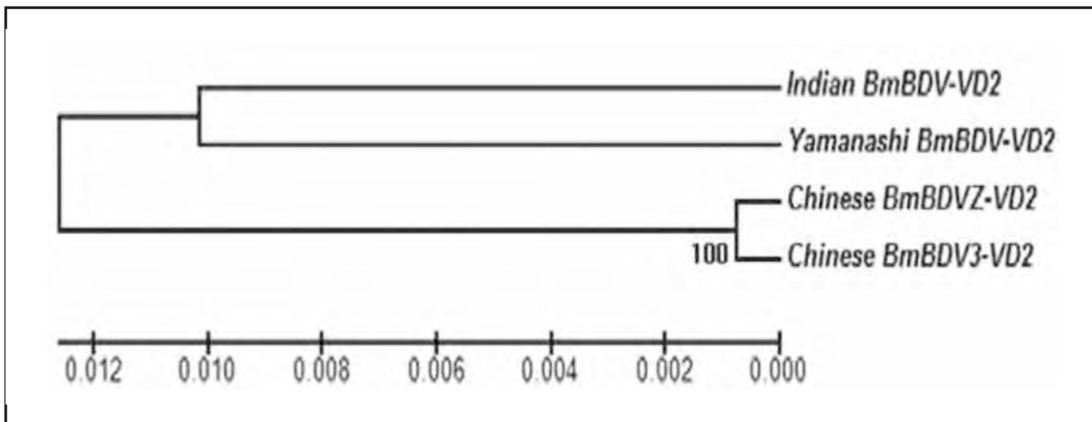


Figure 16 : Phylogenetic tree of VD2 DNA segment of Indian isolate of BmBDV

Table 2 : Comparison of genome and the predicted ORFs of the Indian isolate of BmBDV with other reported isolates

	Indian BmBDV	Japanese BmBDV	Chinese BmBDV	BmBDV Z
VD1 length (bp) Accession no.	6542 **KX760110**	6542 **AB033596**	6543 **DQ017268**	6543 **EU623082**
VD2 length (bp) Accession no .	6023 **KX779526**	6031 **S78547**	6022 **DQ017269**	6024 **EU623083**
VD1 ORF1	310>690	310>690	311>691	311>691
VD1 ORF2	473>1423	473>1423	474>1424	474>1424
VD1 ORF3	1416>2915	1416>2915	1417>2916	1417>2916
VD1 ORF4	2939<6286	2939<6286	2940<6287	2940<6287
VD2 ORF1a	684>4163	675>4157	667>1584	668>4150
VD2 ORF1b		1572>4148		
VD2 ORF2	4769<5437	4769<5437	4761<5429	4763<5431
VD1 5' ITR	1>224	1>224	1>224	1>224
VD1 3' ITR	6318<6542	6319<6542	6320<6543	6320<6543
VD2 5' ITR	27>418	1>537	1>524	1>526
VD2 3' ITR	5578<6023	5494<6031	5486<6022	5499<6024

Table 3 : Comparison of ORFs and ITRs between Indian and Japanese (Yamanashi) BmBDV

BmBDV (Japanese isolate)				BmBDV (Indian isolate)				Indian isolate compared with Japanese isolate					
								Nucleotide (bp)			Amino acid residue (aa)		
ORFITRs	Position(bp)	Length (aa)	MW (KDa)	ORF ITRs	Position (bp)	Length (aa)	MW (KDa)	Substi-tution	Inser-tion	Dele-tion	Substi-tution	Inser-tion	Dele-tion
VD1 ORF1	310>690	126	14.0	VD1 ORF1	310>690	126	13.9	5	0	0	1	0	0
VD1 ORF2	473>1423	316	36.5	VD1 ORF2	473>1423	316	36.5	11	0	0	1	0	0
VD1 ORF3	1416>2915	499	54.9	VD1 ORF3	1416>2915	499	54.9	30	0	0	14	0	0
VD1 ORF4	2939<6286	1115	128.2	VD1 ORF4	2939<6286	1115	128.3	45	0	0	15	0	0
VD1 ITRs	1>224	-	-	VD1 ITRs	1>224	-	-	5	0	-	-	-	-
	6319<6542	-	-		6318<6542	-	-	4	1	-	-	-	-
VD2 ORF1	675>4157	1160	133.0	VD2 ORF1	684>4163	1159	132.6	54	0	0	22	0	1
VD2 ORF2	4769<5437	222	27.1	VD2 ORF2	4769<5437	222	27.1	14	0	0	5	0	0
VD2 ITRs	1>537	-	-	VD2 ITRs	27>418	-	-	11	26	38	-	-	-
	5494<6031	-	-		5578<6023	-	-	17	0	38	-	-	-

Table 4 : Comparison of ORFs and ITRs between Indian and Chinese (BmBDV-3) BmBDV

BmBDV (Chinesde isolate)				BmBDV (Indian isolate)				Indian isolate compared with Chinese isolate					
								Nucleotide (bp)			Amino acid residue (aa)		
ORFITRs	Position(bp)	Length (aa)	MW (KDa)	ORF ITRs	Position (bp)	Length (aa)	MW (KDa)	Substi-tution	Inser-tion	Dele-tion	Substi-tution	Inser-tion	Dele-tion
VD1 ORF1	311>691	126	14	VD1 ORF1	310>690	126	14.0	7	0	0	1	0	0
VD1 ORF2	474>1424	316	36.5	VD1 ORF2	473>1423	316	36.5	11	0	0	1	0	0
VD1 ORF3	1417>2916	499	54.9	VD1 ORF3	1416>2915	499	54.9	45	0	0	16	0	0
VD1 ORF4	2940<6287	1115	128.2	VD1 ORF4	2939<6286	1115	128.3	63	0	0	11	0	0
VD1 ITRs	1>224	-	-	VD1 ITRs	1>224	-	-	6	0	0	-	-	-
	6320<6543	-	-		6319<6542	-	-	5	1	0	-	-	-
VD2 ORF1a	667>1584	305	35.6	VD2 ORF1	684>4163	1159	132.6	14	0	1	4	0	0
VD2 ORF1b	1572>4148	858	98					-	-	1	-	-	-
VD2 ORF2	4761<5429	222	27.1	VD2 ORF2	4769<5437	222	27.1	13	0	0	6	0	0
VD2 ITRs	1>524	-	-	VD2 ITRs	27>418	-	-	16	26	24	-	-	-
	5486<6022	-	-		5578<6023	-	-	22	0	24	-	-	-

Table 5 : Comparison of ORFs and ITRs between Indian BmBDV and BmBDV-z

BmBDV - z				BmBDV (Indian isolate)				Indian isolate compared with BmBDV - z					
								Nucleotide (bp)			Amino acid residue (aa)		
ORFITRs	Position(bp)	Length (aa)	MW (KDa)	ORF ITRs	Position (bp)	Length (aa)	MW (KDa)	Substi-tution	Inser-tion	Dele-tion	Substi-tution	Inser-tion	Dele-tion
VD1 ORF1	311>691	126	13.9	VD1 ORF1	310>690	126	13.9	5	0	0	1	0	0
VD1 ORF2	474>1424	316	36.5	VD1 ORF2	473>1423	316	36.5	11	0	0	1	0	0
VD1 ORF3	1417>2916	499	54.8	VD1 ORF3	1416>2915	499	54.9	30	0	0	14	0	0
VD1 ORF4	2940<6287	1115	128.2	VD1 ORF4	2939<6286	1115	128.3	45	0	0	15	0	0
VD1 ITRs	1>224	-	-	VD1 ITRs	1>224	-	-	6	0	0	-	-	-
	6320<6543	-	-		6319<6542	-	-	5	1	0	-	-	-
VD2 ORF1	668>4150	1160	133.1	VD2 ORF1	684>4163	1159	132.6	54	0	0	22	0	1
VD2 ORF2	4763<5431	222	27.1	VD2 ORF2	4769<5437	222	27.1	14	0	0	5	0	0
VD2 ITRs	1>526	-	-	VD2 ITRs	27>418	-	-	17	26	26	-	-	-
	5499<6024	-	-		5578<6023	-	-	22	-	26	-	-	-

AIT 3544 : Validation of Vitellogenin Receptor (VgR) Gene Expression Levels as Molecular Indicator for Fecundity and Fertility in Silkworm Races. (In Collaboration with CSGRC Hosur)

Duration : May 2014 – Dec 2016

G. Ravikumar and Ashok Kumar [CSGRC, Hosur]
Mrs Chitra Manoharan and Mrs. Dyna Susan Thomas, [Research Fellow]

Objectives: To develop a molecular tool to identify high yielding breeds of silkworm using VgR gene in terms of fecundity and fertility.

Insect vitellogenin receptor (VgR) belongs to the low-density lipoprotein receptor (LDLR) gene superfamily and play a critical role in oocyte development by mediating endocytosis of the major yolk protein, vitellogenin (Vg). In our earlier study we found that the vitellogenin receptor (VgR) gene has direct involvement in the production of viable eggs in silkworms and was further validated in the present project.

Using quantitative real time PCR, the present study determined the mRNA expression levels of VgR gene among different races of silkworm, *Bombyx mori*. *VgR* gene expression levels were studied among 17 mulberry silkworm genetic stocks, in which high transcripts levels were observed in BBE0213, BB10255, BBE0293, BB10290, BBE0244, BB1291, BBE0247, and BBI0325 among bivoltine strains and in a multivoltine strain, BMI007. These breeds are high yielders in terms of fecundity (>500) whereas the rest 8 low yielders (<500) showed lower *VgR* expression levels. The differential expression levels of *VgR* in silkworm races were found to be correlated with that of fecundity, high *VgR* transcripts leads to high fecundity. The highest expression level was seen in BBE0213 in which the fecundity is reported to be 590 followed by BBI0255 whose fecundity is 572 (Fig 17).

These results are consistent with our earlier observations on the impact of *VgR* gene on fecundity and fertility of silkworms. Further, validation of the *VgR* gene through this study strongly indicates its use as a functional marker in screening promising silkworm races for the selection of high yielders.

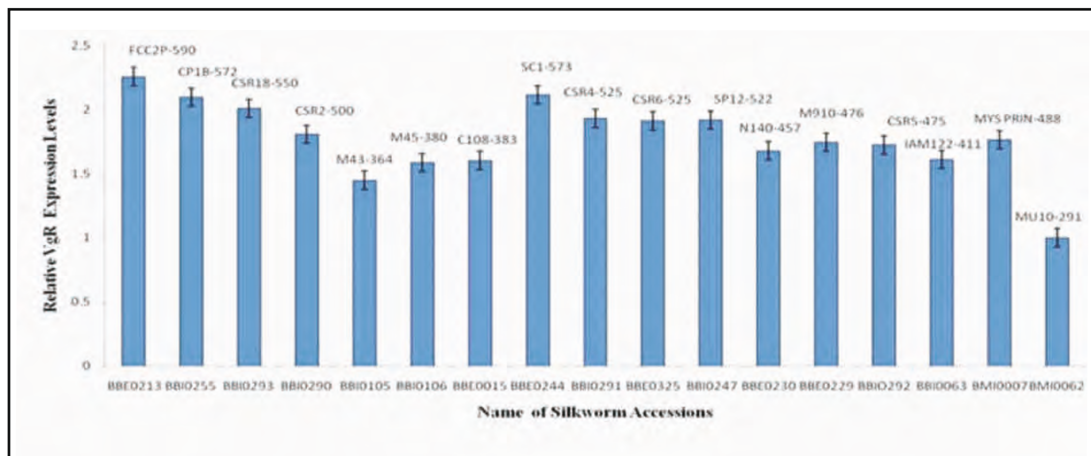


Figure 17: Taqman Real Time PCR analysis of VgR gene expression of silkworm strains. The name of the silkworm accession (Lower), its common name and fecundity (Upper) are indicated in each histogram. Values represent mean ± SE.

AIT 3468 (DBT) : Development of RNA interference (RNAi) based Nuclear Polyhedrosis Virus resistant transgenic silk moth (Jointly with CDFD, Hyderabad and APSSRDI, Hindupur under Center of Excellence for Genetics and Genomics of Silk moth to Center for DNA Fingerprinting and Diagnostics, Hyderabad)

Duration : Sep 2011 – Mar 2017

Awasthi A K, A. R. Pradeep
Mrs. Varada Burdekar [JRF]

Rearing performance of transgenic larvae of BC4F16 – BC4F19 generations

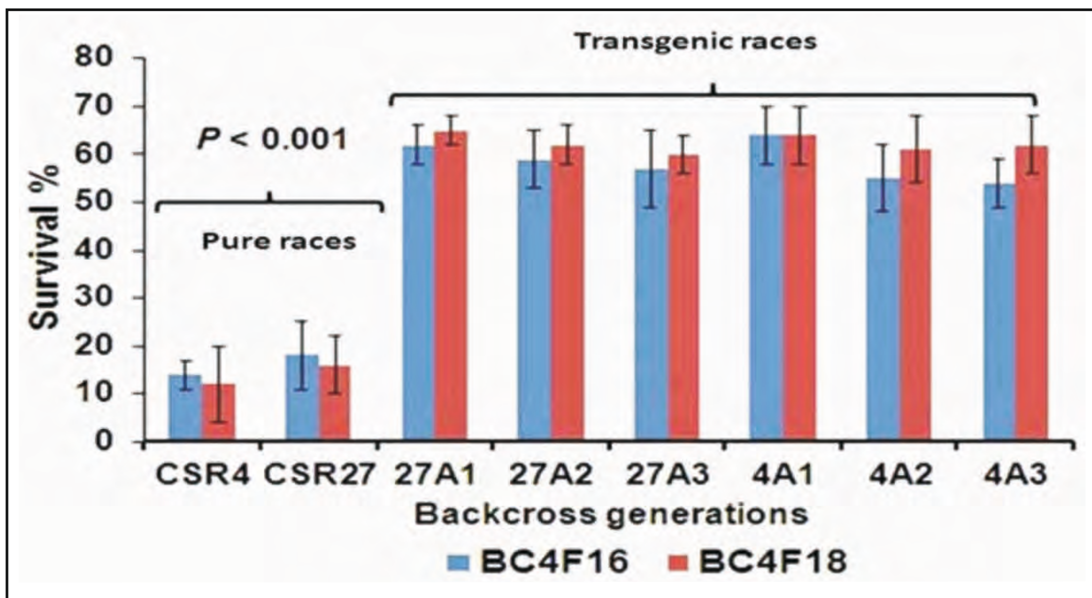
BmNPV is one of the most devastating microbial parasite that causes upto 50% loss in Indian sericulture. Conventional breeding techniques are employed to develop NPV tolerance in high yielding silkworm strains. However, the NPV multiplication rate is at a higher rate and the parasite prevailed. In order to develop NPV resistance in silkworm races, RNA interference (*RNAi*) technique is employed by which double stranded (ds) RNA has been incorporated into the Nistari strain of *B. mori* through piggybac vector- mediated germline transgenesis (Subbaiah et al (2013) Genetics, (193: 63–75). These authors found that the transgenes are integrated in the silkworm genome and were mapped in different chromosomes which facilitate the transfer of

transgenes from one generation to the next. In order to increase the NPV tolerance in high yielding CSR4 and CSR27 races, crosses were made between transgenic pure races with CSR4 and CSR27 and developed F1 generation. The F1 moths were back crossed with CSR4 / CSR27 parent for four generations (BC4) followed by sibmating among the BC4 individuals (BC4F1) and was continued for 18 generations (BC4F18). In the year 2016-17, BC4F16 to BC4F19 were developed. Survival rate after NPV infection and yield traits of the four generations were noted.

The silkworm rearing to introgress transgenes in recipient lines have been continued to BC4F19 generations. Larvae of BC4F16 generation were infected with NPV polyhedra (20000 PIB / larva) and the mortality and infection symptoms were recorded. Larval weight of NPV- infected worms was reduced significantly ($P<0.01$) in CSR27- crosses compared with CSR4 crosses. Survival after NPV infection remained at 56% in both groups (Fig. 18). Survival rate of transgenic lines were tested after exposure of BC5F18 generation larva to NPV. The transgenic CSR27 and transgenic CSR4 lines showed survival up to 65%. Moths were examined for presence of “transgene reporter, red fluorescence” in ommatidia through visual markers (RFP). Out of the moths tested, 99% were with RFP and few were identified as without red fluorescence.

BC4F17 and BC4F18 generations showed significant increase in larval and pupa weight in transgenic lines compared to normal pure lines of CSR4 and CSR27. The shell weight did not show significant variation from normal CSR races indicating that yield characters in transgenic lines attained that of pure race

Mature larval weight of CSR4 and CSR27 were 4.58 ± 0.31g and 4.75 ± 0.24g respectively whereas in the BC4F19 generation, mean larval weight was 4.3 ± 0.22g for transgenic CSR27 and CSR4 larvae. The cocoon weight of CSR27 was 2.19 ± 0.056g with shell ratio of 23% whereas that of transgenic CSR27 has 1.94 ± 0.07g with 20.13% shell ratio (Fig 19**)**. The DFLs prepared were preserved for four months and six months hibernation schedule. Transgenic CSR4 and CSR27 were developed with 45-50% survival after NPV infection. Reduction in survival and yield traits in BC4F19 generation may be due to the effect of increased environmental temperature during summer. Moths of the transgenic CSR4 and CSR27 lines were independently kept for sibmating and egg layings. The disease- free layings (DFLs) were preserved at specific temperature schedule for four and six months hibernation schedule for maintenance.



by back cross breeding of CSR4 and CSR27 with transgenic Nistari lines in comparison with parental races

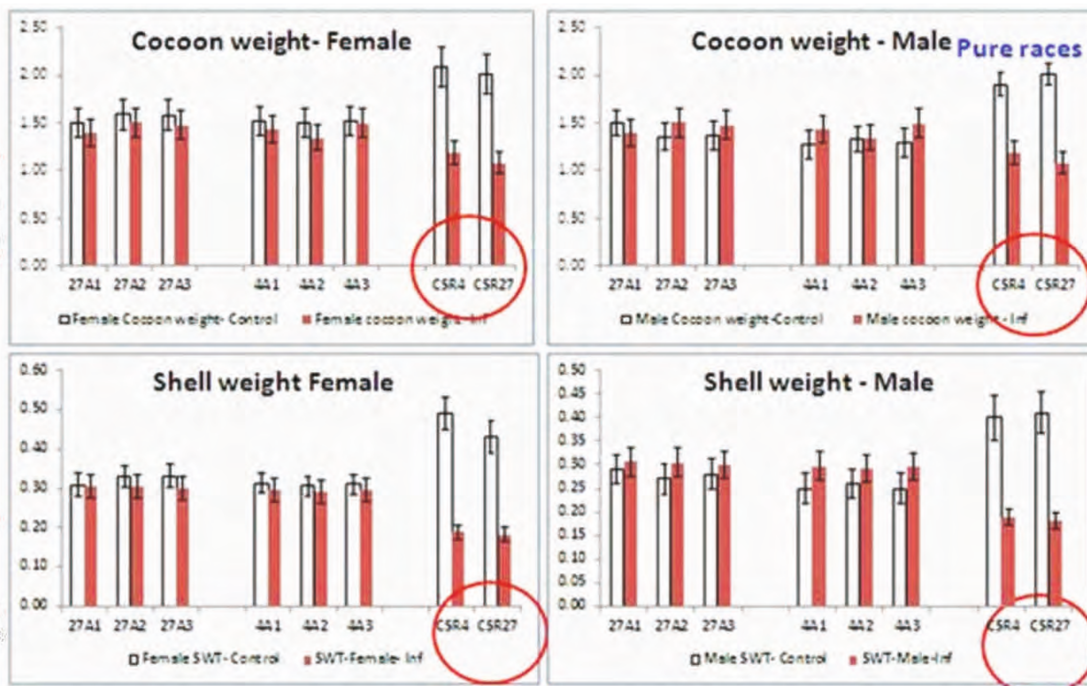


Figure 19 : Yield traits of transgenic silkworms of BC4F16 and BC4F18 generations developed by back cross breeding of CSR4 and CSR27 with transgenic Nistari lines in comparison with parental races (circled)

AIT 3494 (DBT) : Host - parasite interaction: Transcriptome responses to parasitism in the silkworm *Bombyx mori.*

Duration : Jan 2013 – July 2016

Pradeep A.R, A. K. Awasthi and K. M. Ponnuvel
Ms. Pooja M. and Ms. Shambhavi P. H. [JRFs]

In sericulture, infestation of silkworm, *Bombyx mori* with the uzi fly, *Exorista bombycis* is rampant. Though mechanical measures to prevent uzi attack are being practiced, if attacked, the larvae will succumb. Infection of another dreaded parasite, microsporidian, *Nosema bombycis* causes the pebrine disease. Though moth examination procedures are continuously practiced to control microsporidian infection, target oriented control of the parasite are not yet identified. Moreover, immune responses of silkworm against different parasites have to be explored to identify immunocompetent strains to use them as parents to synthesize new strains with larger tolerance against parasites. The project is aimed to identify the molecular responses of *B. mori* against the infection of *E. bombycis* and *N. bombycis*.

Detailed analysis on the impact of parasitism in *B. mori* showed infection- induced modulation in gene expression, induction of cytotoxicity, activation of detoxification mechanisms and immune reactions. In the present study, few important molecules that are closely associated with the parasite interactions were identified. These include heme agglutination factor, *humoral lectin* and lipid transporter cum recognition protein, *apolipophorin*. Most of the immunity – associated genes showed suppressed activity after microsporidian infection. Two important mechanisms like toll pathway activation and melanization showed varied responses. Toll pathway was activated in the later stages of microsporidian infection and the melanization is suppressed in the later stage.

Present investigation for the first time showed cytotoxicity induced by a dipteran parasitoid larva (uzi fly, *Exorista bombycis)* in any host insects. Signs of cytotoxicity as well as activation of detoxification genes and mechanisms in haemocytes unambiguously showed that the host immune system is suppressed by inducing toxicity in hemocytes by *E. bombycis* larva. Due to the infection, level of reactive oxygen species (RoS), H_2O_2 was increased and in synchronization with increased lipid peroxidation, degranulation of structured granules and porosity of plasma membrane of hemocytes revealed that the toxicity is caused by increase in H_2O_2 elicited by *E. bombycis* invasion. Moreover microarray showed upregulation of several genes associated with detoxification mechanisms and down regulation of immune genes, molecular functions and other biological processes **(Fig. 20)**

Infection of *B. mori* larvae with *Nosema bombycis* (microsporidian) induced expression of an exclusive protein band of 55kDa in haemolymph at 8th day and180kDa size in hemocytes on 11th day after infection. Mass spectrometry of protein band resolved from hemocytes by 10% SDS-PAGE showed enhanced expression of few proteins viz, hemocytin, antichymotrypsin, chymotrypsin inhibitor, prophenol oxidase 2 (PPO2), apoplipophorin and arylphorin. These proteins are associated with immune activity such as serine protease inhibition, cell adhesion and parasitic recognition.

Analyses of mass spectrometry data showed presence of ten uncharacterized proteins which are predicted to have different functional roles (Table 6). Two uncharacterized proteins among the ten showed putative functions of protein binding (H9JAZ8) and lipid transporter activity (H9JJ98).Kyte - Dolittle hydropathy plots prepared from the amino acid sequence of the accessions H9JJ98 and H9JAZ8 showed large number of hydrophilic amino acids with several negative peaks which further indicates the presence of globular proteins.On similarity search using BLASTP, H9JAZ7 showed close similarity with hemocytin and its isoforms of lepidopteran insects. The accession H9JJ98 showed similarity with uncharacterized protein from different insects. During the search, NCBI-conserved domain (CDD) identified three conserved domains viz von Willebrand factor (VWD), cysteine 8 (C8) and trypsin inhibitor like (TIL) from H9JAZ8whereas epidermal growth factor like (EGF) was also identified by PROSITE. From H9JJ98, three conserved domains, Vitellinogen (superhelical), lipid transport protein (N- terminal) and VWD factors were identified. Present observations showed functional activity of the uncharacterized proteins induced after the microsporidian infection. Modeling and simulations of the uncharacterized proteins showed presence of disuplhide bonds in both proteins (Fig. 21)

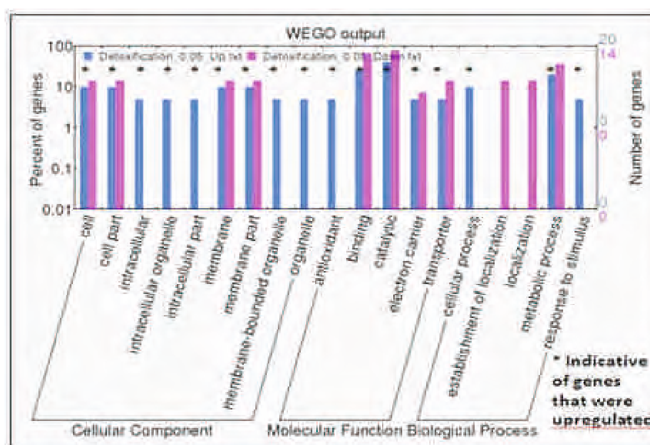


Figure 20: WEGO output showing the functional genes that were upregulated or down regulated in hemocytes of *B. mori* larva after infestation by uzifly, *Exorista bombycis*.

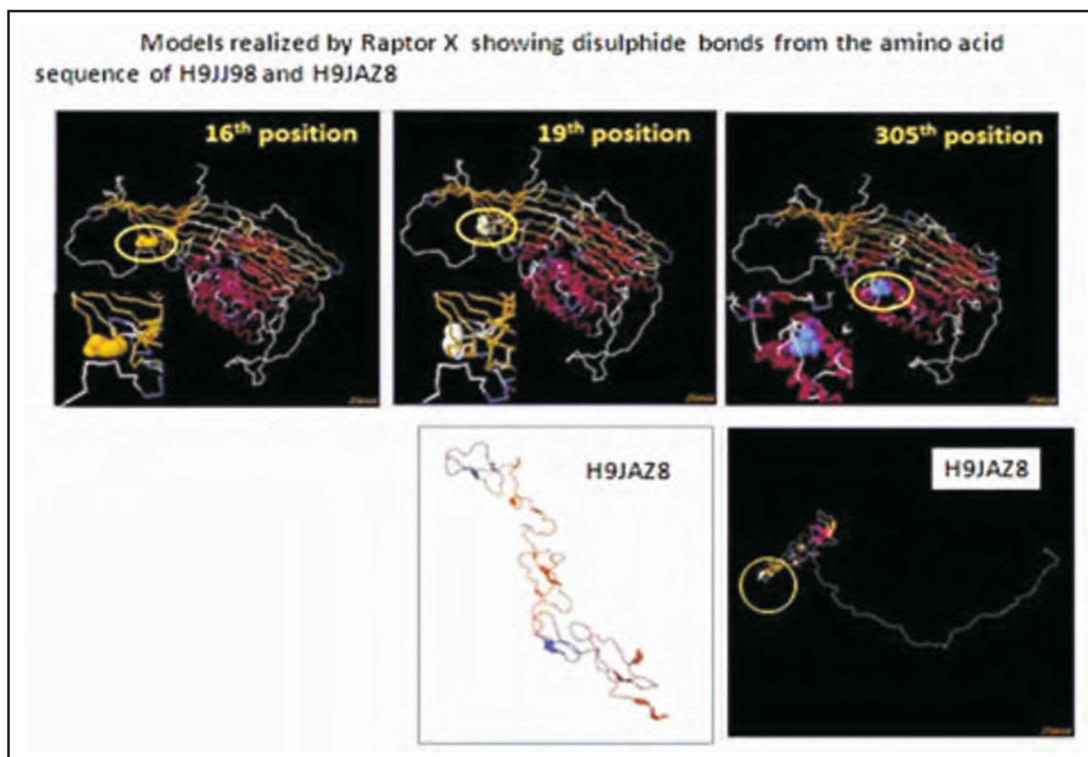


Figure 21 : Protein models realized by Raptor X showing disulphide bonds from the amino acid sequence of two uncharacterized proteins H9JJ98 and H9JAZ8

Table 6 : Protein models realized by Raptor X showing disulphide bonds from the amino acid sequence of two uncharacterized proteins H9JJ98 and H9JAZ8

S.No.	Protein accession	Name	Putative function
1.	Uncharacterized protein (H9JCP0)	Ommochrome binding protein like	Involved in coloration and detoxification pathways by transporting tryptophan metabolites and increase in haemolymph concentration prior to diapause
2.	Uncharacterized protein (H9JP12)	Sex specific storage protein - 1 (Hemoyanin domain)	Hemocyanin an important antigen non-specific immune protein.
3.	Uncharacterized protein (H9JH62)	Basic juvenile hormone suppres-sible protein - 2	Role in hormonal regulation

4.	Uncharacterized protein (H9J236)	Atlastin like	Member of the dynamin protein superfamily and it can mediate homotypic fusion of endoplasmic reticulum (ER) membranes, which is required for many biological processes.
5.	Uncharacterized protein (H9J8B0)	ripeptidyl-peptidase T 2 isoform X1	A serine peptidase involved in antigen processing, cell growth, DNA repair, and neuropeptide mediated signaling.
6.	Uncharacterized protein (H9JFE1)	Plexin A3	Combining with a semaphorin, and transmitting the signal from one side of the membrane to the other to initiate a change in cell activity.
7.	Uncharacterized protein (H9JJ98)	Transmembrane protein	Lipid transporter
8.	Uncharacterized protein (H9JDB5)	UDP-glucose: glycoprote in glucosyltransferase	Glucosyltransferase activity
9.	Uncharacterized protein (H9JV11)	Uncharacterized protein	Actin binding activity
10.	Uncharacterized protein (H9JAZ8)	Uncharacterized	Protein binding

BRAIN STORMING SESSION ON SERIBIOTECHNOLOGICAL INTERVENTION IN SERICULTURE

A brain storming session participating different research Institutes of CSB was convened on 28th January 2017 at the institute which was chaired by of Prof. H.S. Savithri, Department of Biochemistry, Indian Institute of Science, Bengaluru and Chairperson, RAC, SBRL. At the outset, Dr. R.K. Mishra, Director, SBRL welcomed all participants from CSRTI- Mysore, CSRTI- Berhampore, CSRTI- Pampore, CSGRC- Hosur, CTRTI- Ranchi and CMERTI- Jorhat. He explained the need of conducting the session following instruction of RCC as well as to identify new projects that have direct bearing on the improvement in sericulture through biotechnological tools.

Prof Savithri explained the necessity of multi Institutional Projects that lead to improvement in sericulture, qualitatively and quantitatively. She invited Professor Upendra Nongthomba, IISc and Dr V Sivaprasad, Director, CSRTI, Mysore for initial remarks. Dr. Upendra explained the necessity to exploit the strength of SBRL through network projects with other CSB Institutes and other relevant agencies. Dr. Sivaprasad emphasized the need of application level projects from SBRL through integrated approach culminating in the synthesis of breeds that have high productivity.

Dr. K M Ponnuvel, Scientist D of SBRL briefed the contributions and achievements of SBRL. New marker- assisted selection (MAS-N) breeds for NPV resistance, development of RNAi- mediated transgenic CSR races through back cross, identification and utility of functional genomics in diapause- induction, vitellogenin expression and identification of high- fecundity races, long duration lines of Nistari with larger cocoon weight, identification and utility of immune genes for identification of immunocompetent strains against different pathogens and parasites, identification of DNV2 pathogens and resistant races, autumn – specific breeds for J & K, molecular identity of ecoraces / populations of *Samia Cynthia ricini, Antheraea mylitta* and *A. assamensis*, multiplex PCR for detection of three pathogens in one reaction, identification of Ifla virus of *A. mylitta*, causative agents for Tiger band disease in *A. pernyi* of Manipur/ Himachal Pradesh were explained. He requested for use of new techniques like CRISPR/CAS9 for genome editing where specific gene is known to contribute largely in future along with Marker assisted selection.

Dr. Girish Naik, Scientist D of CSRTI presented the achievements in mulberry genomics and suggested to improve the mulberry varieties through identification of

disease resistance, leaf yield output, development of mapping population and utility in converting leaf protein to silk protein. Dr. Mantira Murthy, Scientist D, CSRTI, Mysore explained utility of antiviral genes like BmNOX, Pure Mysore race- specific markers, markers associated with different yield traits and thermo tolerance from *B. mori.*

Dr Saumen Chattopadhyay, Scientist D, CSRTI, Berhampore emphasized the need of study on white fly infecting mulberry causing 26% crop loss, water deficient stress tolerant variety, marker- assisted selection of high temperature and high humidity tolerant varieties. Moreover he emphasized the need of early detection of Pebrine and BmNPV that are essential to prevent the spreading of the pathogens. On white fly infestation on mulberry at Berhampore, the house suggested to take up an initial assessment on resistant and susceptible varieties, identify phenotypes before molecular variation studies on resistance.

Dr Pawan Shukla, Scientist B from CSRTI, Pampore requested the need to investigate cold tolerant mechanism in Leh, Laddakh and Gurej regions, to develop mulberry varieties with biotic and abiotic stress tolerance, high leaf yield and palatable leaf and disease resistant silkworm hybrids, genome sequences of NPV isolates from north India and diagnostic kit for rapid detection of diseases and identify autumn-specific breeds.

Dr Geetha N Murthy, CSGRC emphasized the need to investigate on molecular Ids for all accessions and creation of a National database. She requested to identify tolerant silkworm races and novel genes to utilize them in improving wild silkworm races and non-sericultural use of silkworm genetic resources.

Dr. Pandey Scientist C from CTRTI, Ranch emphasized that Tasar industry requires new breeds for high disease resistance and high yield and control of pebrine disease. Dr. Sivaprasad suggested to have close monitoring on pebrine detection and spreading in north India through pebrine monitoring committee and immediate disposal on detection.

The house suggested initiating collaboration with SBRL on the following areas:

1. Mulberry – related research with CSRTI, Mysore as the centre of activity.
2. Disease resistance and pebrine control in non-mulberry sector with SBRL as centre for molecular analysis.
3. To develop methods for early detection of microsporidian spores in mulberry sericulture to avoid spreading and multiplication in north India under collaboration with CSRTI, Berhampore.

4. Development of new breeds for NPV resistance suitable to northern India under collaboration with CSRTI, Berhampore and SBRL.
5. Developing database on mulberry and silkworm research as a project at national level
6. Induction of diapause in muga silkworm- in collaboration with CMERTI, Jorhat.
7. Identification of beneficial microorganisms associated with mulberry and non-mulberry silkworms for its utilization in silkworm improvement through better leaf conversion.

Silkworm Stock maintenance

1. Six lines of transgenic CSR4 and CSR27
2. Silkworm breeds for NPV resistance developed through marker assisted selection (MAS-N 4, MAS-N 6 and MAS-N7).
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/CONFERENCES/WORKSHOPS

As part of capacity building program in this institute 21 students belonging to different colleges were given 1-6 months training in the molecular biology techniques and the project report have been submitted to the respective Universities.

CENTRAL SEED ACT IMPLEMENTATION

Dr. A. Ramesha and Dr. K.S. Tulsi Naik attended a short term training organized by NSSO, Bangalore from 04.01.2017 to 06.01.2017 at SSPC Bangalore in Quarantine procedures as a part of implementation of the provisions of Central Silk Board(Amendment) Act 2006.

Under the Seed Act implementation program, Dr. A. R. Pradeep, Scientist D, Dr. G. Ravikumar, Scientist D and Dr. K. M. Ponnuvel, Scientist D were attended duty of Seed Analysts in Sericultural belt in Southern Karnataka.

CONFERENCE

Dr. K.S. Tulsi Naik attended five days “**Programme on Integrated Scientific Project management for women Scientists and Technologists**” sponsored by DST, Govt. Of India from November 21 to 25, 2016 at the Centre for Organization Development (COD), Hyderabad.

OVERSEAS VISIT

Dr. G. Ravikumar, Scientist-D was deputed by Central Silk Board to Transgenic Silkworm Research Unit, National Agriculture Research Organization (NARO), Tsukuba, Japan for undergoing training in **Microinjection technique for developing transgenic silkworms** from January 12-16, 2017

PUBLICATIONS

1. Pooja Makwana, Pradeep AR, Shambhavi. PH, Ponnuvel KM and Trivedi K (2017) The dipteran parasitoid *Exorista bombycis* induces pro- and anti-oxidative reactions in the silkworm *Bombyx mori* : Enzymatic and genetic analysis: **Archives of Insect Biochemistry and Physiology** 94(2):e21373.

2. Rajni Bala, Ulfath Saba, Meenakshi Varma, Dyna Susan Thomas, Deepak Kumar Sinha, Guruprasad Rao, KanikaTrivedy, Vijayan Kunjupillai and Ravikumar Gopalapillai (2016). Cloning and Functional Characterization of a Vertebrate Low-Density Lipoprotein Receptor Homolog from Eri Silkmoth, *Samia ricini.* **Journal of Molecular Biochemistry** 5: 87-94.

3. Dyna Susan Thomas, Chitra Manoharan, KanikaTrivedy, Kunjupillai Vijayan and Ravikumar Gopalapillai (2016). Lipophorin and its Immunological Properties of Eri Silkmoth, *Samia ricini*. **Sericologia** 56:74-83.

4. Pradeep ANR, Asea A, Kaur P (2016) Nucleolin Transports Hsp72 to the Plasma Membrane Preparatory to its Release into the Microenvironment. **Journal of Cell Science & Therapy** 7: 254. doi: 10.4172/2157-7013.1000254

5. Lekha G, Gupta T, Vijayagowri E, Awasthi AK, Ponnuvel KM (2016). Genome – wide identification, characterization of sugar transporters genes in the silkworm *Bombyx mori* and role in *Bombyx mori* nucleopolyhedrovirus (BmNPV) infection. **Gene** 579, 162- 171

6. K.M.Ponnuvel, S. Sasibhushan, E.Vijaya Gowri, Geetha N.Murthy and A.K. Awasthi (2016) . Expression of paralytic peptide binding protein (PP-BP) gene during Induction of Egg Diapause and its multi-gene organization in Silkworm *Bombyx Mori*. **Sericologia** 56(1): 49-53,

7. Wazid Hassan, B. Surendra Nath and Geetha N. Murthy (2016) Comparative phylogenetic analysis of intergenic spacers and small subunit rRNA gene sequences of two microsporidian isolates from *Antheraea myllita.* **African Journal of Biotechnology.**15(47): 2678-2686

8. Wazid Hassan and B. Surendra Nath (2016) Sequence and phylogenetic analysis of Intergenic spacer (IGS) region of ten microsporian isolates infecting Indian vanya silkworms (*Samia cynthia ricini* and *Antheraea assamensis*). **Int. J. Indust. Entomol**. 33(2) 121-131

Following research scholars of SBRL were awarded PhD from University of Mysore

1. **Dr. G. LEKHA** : Topic - Differential level of gene expression in silkworm associated with resistance to *Bombyx mori* nucleopolyhedrovirus (BmVPV) - Guide: Dr. K.M.Ponnuvel

2. **Dr. G. BHUVANESWARI** : Topic - Molecular characterization and phylogenetic relationship of microsporidians species infecting various mulberry insect pest: Studies on cross infectivity to silkworm, *Bombyx mori* L – Guide: Dr. B. Surendranath

3. **Dr. WAZID HASSAN** : Topic - Genetic diversity and phylogenetic relationships among microsporidia infecting non-mulberry silkworms - Guide: Dr. B. Surendranath

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.	Japanese Society for the promotion of Science, (JSPS) Tokyo, Japan
4.	IISc, Bengaluru
5.	IIT, Guwahati
6.	Dept. Of Genetics, University of Delhi , South Campus
7.	CDFD, Hyderabad
8.	APSSRDI, Hindupur
9.	M/s. Unilever Industries, Bengaluru
10.	C-CAMP, Bangalore
11.	NIMHANS, Bangalore
12.	SSTL, Kodathi, Bangalore
13.	RSRS, Kodathi, Bangalore
14.	NSSO, Bangalore
15.	CSTRI, Bangalore
16.	CSR&TI, Mysore
17.	CSR&TI, Berhampore, West Bengal
18.	CSGRC, Hosur
19.	CTR&TI, Ranchi
20.	CMER&TI, Lahdoigarh
21.	CSR&TI,Pampore
22.	BTSSO, Bilaspur

RESEARCH ADVISORY COMMITTEE

Sl.No.	List of RAC Members	Remarks
1.	**Prof. H.S. Savithri** Dept. of Biochemistry, Indian Institute of Science Bengaluru – 560 012	**Chairperson**
2	**Prof. Usha Vijayaraghavan** Dept. of Microbiology & Molecular Biology Indian Institute of Science Bengaluru – 560 012	**Member**
3	**Dr. R. Ashokan** Principal Scientist Dept. of Biotechnology IIHR, Hesaraghatta, Bengaluru – 560 089	**Member**
4	**Prof. P.H. Ramanjini Gowda** Professor Dept. of Biotechnology, UAS, GKVK, Bengaluru - 560 065	**Member**
5	**Prof. H.E. Shashidhar** Professor Dept. of Biotechnology, UAS, GKVK, Bengaluru-560 065	**Member**
6	**Dr. Arunkumar K. P** Staff Scientist & Group Head, Lab of Molecular Genetics Center for DNA Fingerprinting & Diagnostics [CDFD] Bldg.7,Gruhakalpa,5-4-399/B, Nampally, Hyderabad - 500 001	
7	**The Director (Tech),** Central Silk Board Ministry of Textiles, Govt. of India CSB Complex, BTM Layout, Madiwala, Bengaluru – 560 068	**Member**

Sl.No.	List of RAC Members	Remarks
8	**The Director** Central Sericultural Research & Training Institute Central Silk Board, Ministry of Textiles, Govt. of India Srirampura, Manandavadi Road, Mysore – 570008	**Member**
9	**The Director** Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore – 742 101 West Bengal	**Member**
10	**The Director** Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Gallandar, Pampore -192121 (J&K)	**Member**
11	**The Director** Central Tasar Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India PO - Piska-Nagri, Ranchi - 835 303, Jharkhand	**Member**
12	**The Director** Central Muga Eri Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of IndiaP.O. - Lahdoigarh, Jorhat – 785 700, Assam	**Member**
13	**The Director** Central Sericultural Germplasm Resources Centre P.B. 44, Thally Road, Krishnagiri District Hosur – 635109, Tamil Nadu	**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

Shri A. H. Hanumatharayyappa was appointed as new Chairman of the Central Silk Board. He visited SBRL on 25-08-2016.

Dr. S. Ayyappan formerly Director – General of ICAR, Chairman, CSB Research Co-ordination Commitee (RCC) visited SBRL on 12-08-2016.

HUMAN RESOURCES

Sl.No.	Name	Designation
1	Dr P.K Mishra	Director 01.09.2016-30.09.2016
2	Dr R K Mishra	Director17.10.2016-12.02.2017
3	Dr. Kalidas Mandal	Director13.02.2017-till date
4	Dr. A. K. Awasthi	Scientist - D & Director I/c (till 31.07.2016)
5	Dr. M. Venkateswarulu	Scientist - D till 10.06 2016
6	Dr. (Mrs.) Geetha N. Murthy	Scientist - D till 31.05.2016
7	Dr. K. M. Ponnuvel	Scientist - D
8	Dr. G. Ravikumar	Scientist - D
9	Dr. A. R. Pradeep	Scientist - D
10	Mr. S. Ramesh Kumar	Scientist - C till 30.05.2016
11	Dr Tulsi Naik K S	Scientist - B
12	Dr Sailaja Bandam	Scientist - B (till 04.08.2016)
13	Dr A Ramesha	Scientist - B
14	Mr. S. N. Gundu Rao	Technical Assistant
15	Mr.Venkateshaiah	Technical Assistant till 25.06.2016
16	Mr. G. Sumant Kumar	Technical Assistant
17	Mr. R N Sreekantaiah	Technical Assistant from 01.06.2016
18	Mr. Rangaiah	Field Asst. from 25.06.2016 till 09.01.2017

RESEARCH FELLOWS

Sl No	Name	Designation	Division
1.	Mr. Wazid Hussain	Senior Research Fellow	Molecular pathology
2.	Mrs. G. Bhuvaneswari	Research scholar	Molecular pathology
3.	Mrs. Lekha G	Junior Research Fellow	Genomics
4.	Mrs Varada Burdekar	Senior Research Fellow	Genomics
5.	Ms Tania Gupta	Junior Research Fellow	Genomics
6.	Mrs Vijaya Gowri	Junior Research Fellow	Genomics
7.	Ms Shambhavi P Hungund	Junior Research Fellow	Proteomics
8.	Mrs Dyna Susan Thomas	Junior Research Fellow	Genomics
9.	Mrs Chitra Manoharan	Junior Research Fellow	Genomics
10.	Mrs. Sandhya Rasalkar	Junior Research Fellow	Genomics

ADMINISTRATION

Sl No	Name	Designation
1	Mrs. M. Jayalakshmi	Asst. Director(A&A)
2	Mr. Mohan Raj	Asst.Supreintendent
3	Mr Chandrashekhar Rao	Asst.Supreintendent
4	Mr A. Mallesha	Asst.technician
5	Mr. S. Nagesh	Driver
6	Mr.Kenchappa	Multi Tasking Staff

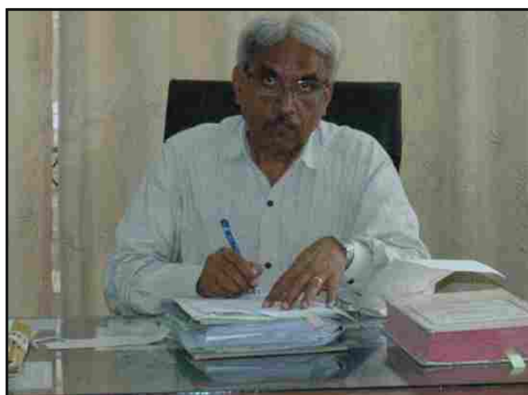
SUPPORTING STAFF

Sl No	Name	Designation
1	Mr. Shankar Shetty	SFW (volunteer retirement 3.09.2016)
2	Mr. L.P Sampangi Rao	SFW
3	Mr.Hombalaiah	SFW
4	Mr M. Krishnappa	SFW
5	Mr N Pillappa	SFW
6	Mr E Shivanna	SFW
7	Mrs. Lakshmamma	TSFW

SUPERANNUATION

Dr. Arvind K. Awasthi, Scientist D and Director (I/c) retired from service of Central Silk Board (Government of India) on attaining superannuation at the age of 60 years on 31 July 2016. Dr. Awasthi's contribution include development of NPV resistant lines and development of transgenic CSR races with higher NPV tolerance through conventional as well as molecular breeding showing application of biotechnological principles in development of sericulture.

The fraternity of SBRL thanking him for his esteemed service and wish him a happy retired life.



Dr. Pradeep Kumar Mishra and Dr. P. Jayaprakash rendered their esteem service as Directors of SBRL during intermittent period in the year. Both Dr. Mishra and Dr. Jayaprakash were Senior Scientists of Central Silk Board and have contributed immensely towards the development of mulberry and non-mulberry sericulture in the country. We, SBRL fraternity thankfully remember the contribution and support extended to this laboratory for improving Seri biotechnological research and wishes them happy retired life.

Dr. P. K. Mishra

Dr. P. Jayaprakash

Financial Progress (2016-17)

During the year under report, an expenditure of Rs.279.59 lakhs was incurred from Grants-in-Aid sanctioned by CSB and Department of Biotechnology, Government of India

Sl No	Particulars	Amount (Rupees in lakhs)
1	Salary and allowances	143.75
2	Wages & EPF	14.33
3	Travelling expenses	2.83
4	Contingent expenses	48.21
5	Assets	29.93
6	Others	32.52
7	Sub Total	271.57
8	DBT assistance	8.022
	Total	**279.59**

EVENTS

International Yoga Day was observed on 21 June 2016 as per instruction of Central Silk Board.

Hindi Fortnight

Hindi fortnight was observed from 1 – 14th September 2016 and conducted workshops for scientists. Various competitions were organised for staff to reveal proficiency in Hindi.

Vigilance Awareness week

Vigilance Awareness week was organized from 31st October to 5th November 2016 as per instruction of Government of India and Central Silk Board.

Swacchta Pakwada

Swacchta Pakwada was organised from 15.05.2016 - 31.05.2016 jointly with Silkworm Seed technology laboratory, (SSTL), Kodathi and various programme was organised as per central office instructions.