

AIB 3480

**Development of silkworm
(*Bombyx mori* L.) breeds from
a gene pool with higher
genetic plasticity.**

Time period

SEPTEMBER, 2012 - AUGUST, 2016

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Central Sericultural Research & Training Institute

Central Silk Board: Ministry of Textiles: Govt. of India

Berhampore – 742 101, Murshidabad, West Bengal,

INDIA

PROFORMA – I

PART I: GENERAL INFORMATION

1. Name of the Institute / University /Organization submitting the Project Proposal:

Central Sericulture Research & Training Institute,
Berhampore – 742101, Murshidabad, West Bengal.

2. Status of the Institute (s):

Institute for Research & Development of Mulberry
And Mulberry Silkworm

3. Name (s) and designation(s) Of the Executive Authority of the Institute / University forwarding the application:

Dr. Kanika Trivedy
Director, Central Sericulture Research
&
Training Institute,
Berhampore - 742101, Murshadabad, West Bengal.

4. Project Title:

Development of Silkworm Breeds (*Bombyx mori* L) from a
Gene Pool with higher Genetic Plasticity.

5. Category of the Project: AIG - 02

6. Specific Area: Silkworm Improvement

7. Duration: 4Years

8. Total Cost: 12.25 Lakhs

9. Is the Project single Institutional or multi- institutional:

Single Institutional

**10. If the Project is multi-institutional, please furnish the following
Name, Designation and Address of the Project Coordinator:**

N.A.

11. SUMMARY OF THE PROJECT PROPOSAL

In this project, six multivoltine (V_3) strains / breeds viz., M.Con.4 (a Congenic multivoltine breed developed through introgression of high cocoon shell from D6p in M6DPC having high survival), Nistari, Pure Mysore (PM), APM-1, Cambodge and C'nichi that are recognized as tolerant with good adaptability (Annexure-III) and six high cocoon shell weight bivoltine (V_2) breeds viz., B.Con.4 (a Congenic bivoltine breed developed through introgression of high survival character in JPNp from M6DPC having high survival), KPGA, RSJ-14, DUN-21, CSR-2 and APS- 45 (Annexure-III) will be collected from different geographical regions having specific climatic variation as follows;

- i. **Highly variable climatic condition in plain region in every rearing season**
- ii. **Congenial climatic region in plain region**
- iii. **Low temperature with low humidity at high altitude**
- iv. **Moderate variable climatic condition**

The aggregation and genetic assimilation will be done through **Convergent crossing** among proposed multivoltine breeds for horizontal resistant and among bivoltine breeds for high cocoon shell weight to bring **Genetic Plasticity** in the gene pool. Thereafter, separate multivoltine and bivoltine lines will be screened having genetic plasticity in the gene pool for a target trait under variable climatic situation. The screened multivoltine and bivoltine breed will be used further to develop **Congenial bivoltine breed for tolerant** and **multivoltine breed for high cocoon shell weight** through introgression following the method proposed by Chattopadhyay *et al.*, 2001 a, b; 2005 a, b; (Annexure-VI). Each and every step of breeding, molecular characterization will be done to understand the diversity or similarity at biochemical and DNA level and its' persistence in developed breeds which may considered as one of the molecular factor / marker for future breeding programme.

PART II: PARTICULARS OF INVESTIGATORS

12. i) **Name:** **Dr. A. K. Verma**
- ii) Date of birth: 28.12.1960
- iii) Sex : Male
- iv) Indicate whether Principal Investigator/ Co-investigator: **Principal Investigator**
- v) Designation & Department: **Scientist - D**
Silkworm Breeding & Genetics Section
- vi) Institute / University Address: **Central Sericulture Research & Training Institute,**
Berhampore- 42101, Murshidabad, West Bengal.

Name: Dr. N. Chandrakant
Date of birth: 10. 08.73
Sex: Male
Indicate whether Principal Investigator / Co-investigator: **Co- Investigator-1**
Designation & Department: **Scientist - B**
Silkworm Breeding & Genetics Section
Institute / University Address: **Central Sericulture Research & Training Institute,**
Berhampore- 742101,Murshidabad, West Bengal.

i) **Name:** N.B.Kar
ii) Date of birth: 1958
iii) Sex : Male
iv)Indicate whether Principal Investigator/ Co-investigator: **Co- Investigator-2**
v) Designation & Department: **Scientist - D**
Reeling & Spinning Section
vi) Institute / University Address: **Central Sericulture Research & Training Institute,**
Berhampore- 42101, Murshidabad, West Bengal.

13. No. of Projects being handled by

Each investigator at present: **Principal Investigator: Four projects**
Co- Investigator: 1- one proposed project as PI
Co- Investigator: 2 - Four

14. Proposed Research Fellows: NIL

ART III: TECHNICAL DETAILS OF PROJECT

15. Introduction

Harada, 1949, 1961; Yokoyama, 1974 used the hybrids for commercial exploitation and its performance has resuscitated sericulture farmers in Japan. Thereafter hybridization breeding approach is followed for qualitative and quantitative improvement in India. For the improvement of sericulture, silkworm breeds are generally used through hybridization process. Previously several attempts are made by so many researchers for the development of new silkworm breed through hybridization. Basically, Indian silkworm breeds or strains have highly heterogenic gene pool. Therefore, the genetic load is more and there is a less chance of genetic assimilation. As a result, the yield contributing parameters are highly variable in a population in the same environment. Due to that, the quantum hybrid vigour achieved is not up to the desired extent and lack of consistency in performance. Beside,

rearing of bivoltine breeds as a P1 to prepare huge number of multivoltine x bivoltine eggs are very difficult for commercial purpose during adverse climatic situation. In eastern India, April to September, the humidity of these regions is 95% or above and the temperature is more than 36°C (rearing room temperature) that affects survival of bivoltine (10-15%). It is well known that multivoltine breeds / strains have much higher germ load tolerance and higher survival but produce low quality silk. On the other hand bivoltine (hibernating) breeds or strains have low germ load tolerance (Chattopadhyay and Chatterjee, 1990) and low survival as its gene pool is the carrier of some susceptible genes for diseases like viral (BmNPV), fungal and bacterial which increase the mortality in homozygous condition (Doira, 1993). A Large number of breeds were evolved in different Research Institute followed by developing (RIL) i.e., recombinant inbreeding line (Dutta, 1984; Dutta *et al.*, 2000, 2000a & b; Raghavendra Rao *et al.*, 2002 a, b & 2003; Singh *et al.*, 2001; Kumaresan *et.al.*, 2003; Rao *et al.*, 2004) and used to raise different hybrid but failed to get popularity for their poor survival from April to September especially in Eastern India.

Use of Near Isogenic line (NIL) in plant system was reported by Waugh and Powell, 1992; Haley, *et al.*, 1994, Martin, *et al.*, 1991, Tanksley and Rick, 1980; Tanksley *et al.*, 1981, 1982 Young *et al.*, 1988; Stam and Zeven, 1981; Zeven *et al.*, 1983; Torigoe *et al.*, 1987 for yield improvement, disease resistant and or qualitative improvement. They suggested that Near Isogenic line (NIL) is suitable for biochemical tools when searching for biochemical or genetic marker/ molecular marker linked to the introgressed locus. Blondon *et.al.*, 1994 stated that Near-Isogenic line (NIL) provides a route for quickly identifying markers linked to a trait of interest. It was also stated that NIL would provide discrete difference in the presence or absence of a target sequence and a small region of flanking DNA. Melchinger, 1996; Michelmore *et al.*, 1991, identified molecular markers linked for disease resistance genes by bulk segregation analysis in plants. Blondon-Adam *et al.*, 1994 stated that SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Zubay, 1986 introduced BH2 gene from B strain mice to A strain mice through development of Near Isogenic line for studying histo-compatibility and serological test for presence of H2 antigen (single gene concept) thus suggesting a possibility to develop a new variety / strain for flesh and disease resistant animal. But earlier Congenic / Near Isogenic approach was not used in any economical insect like silkworm.

Later Chattopadhyay *et al.*, 2001 a, b developed a breeding method for introgression of multigenic trait to develop congenic bivoltine breed for high survival and multigenic breed for high cocoon shell weight. It has been observed that these developed breeds are providing higher sustainable Index through out the year in West Bengal situation specially during P1 rearing in comparison to the breeds developed through conventional breeding.

The Congenic hybrids viz., M.Con.4 x B.Con.4 / B.Con.1, M.con.1 x B.Con.1 / B.Con.4, M.Con.1 x M.Con.4 and Nistari x M.Con.4 and its reciprocal showed better performance at VII National level Mulberry Silk worm Hybrid Authorization Programme (MSRAP).

To get more success, it has been proposed further to adopt another breeding approach to bring **Genetic plasticity** in survival (horizontal resistant) and cocoon shell weight in the Gene pool through aggregating genes from well-adapted different strains from different zones and selection under adverse climate to raise multivoltine and bivoltine silkworm breeds better than earlier having **Genetic Plasticity**. Nylin and Gotthard, 1998; Robert *et al.*, 1997; Ballare, 1998; Falconer, 1990; Steams et al., (1991); Steams and Kaweckii, 1994 suggested that there is a possibility to get a good part of the molecular circuitry through **Genetic plasticity, that allows acclimating to fluctuations in a given environmental factor**.

15.1 DEFINITION OF THE PROBLEM:

North and North Eastern region with specially reference to West Bengal, Silkworm rearing is practiced five times in a year at farms and farmer's level due to availability of huge mulberry leaves for high rainfall and fertility of soil. But, the practice of bivoltine rearing or productive silkworm breeds or their hybrids is very restricted for highly fluctuating climatic situation. The favourable climate is from **November to March** and unfavourable is from **April to September**. It has been observed that rearing of bivoltine breeds as P1 to prepare multi x bi hybrid dfls for three commercial rearing (**June- July, August-September and October - November**) is not successful as rearing of P1 bivoltine for preparation of multi x bi hybrids dfls, fall under unfavourable season [**high temperature (> 35^o C) and high humidity (> 85- 90 %)**] causing severe mortality due to grassarie disease.

Therefore, farmers are forced to restrict their rearing only with Nistari (N+p) - the indigenous multivoltine (V₃) strain having maximum horizontal tolerant potentiality both at P1 and in commercial level resulting poor quality silk during the adverse months. Now a day multivoltine hybrid Nistari x M12W is being widely reared at commercial level (**June-July, August-September**) but quality and grading of silk is not improved. The P1 bivoltine rearing in **December and February** for preparing multi x bi or bi x bi hybrid for two commercial rearing viz., **February – March (Falguni) and March – April (Baishakhi)** falls under favourable condition and have no problem except for low temperature in December.

To overcome the P1 bivoltine rearing problems, to reduce the mortality in bivoltine breeds and also to increase the production of multivoltine with quality silk, **Congenic-Breeding** approach has been adopted in silkworm breeding (Annexure-VII) to developed a few multivoltine Congenic breeds (M.Con.1 & M.Con.4) for high cocoon shell weight and bivoltine Congenic breeds (B, Con.1 & B. Con.4) for high survival (higher pupation percentage). These Congenic breeds show higher sustainable index (**SI**) through out the

year as a P1 in West Bengal situation than other normal breeds. The Congenic hybrids viz., M.Con.4 x B.Con.4 / B.Con.1, M.Con.1 x B.Con.1/ B.Con.4, M.Con.1 x M.Con.4 and Nistari x M.Con.4 showed better performance at VII National level Mulberry Silk worm Hybrid Authorization Programme (MSRAP).

Keeping in mind the **very rapid change in the global climatic situation, to combat this change** and to get more success in silk production, it is crucial part of research to bring **Genetic Plasticity in the Gene pool of silkworm**. Therefore it is proposed to try further to adopt another breeding approach to assimilate a number of favourable genes for a trait to bring genetic plasticity in the gene pool through aggregating the gene(s) from well-adapted different strains from different geographical zone of India and selection under adverse climate. So, there is a chance to bring capacity for adaptation caused by genetic changes *i.e.*, **Genetic plasticity**. Thereafter congenic breeding approach will be adopted to develop tolerant bivoltine and productive multivoltine breed with quality silk respectively suitable for variable climatic situation. Further, developed congenic breeds will be use for identifying diversified region in biochemical and DNA level as congenic breed provides a root of quickly identifying marker linked to traits.

In the earlier project, multivoltine and bivoltine convergent gene pool have been developed with genetic plasticity for high shell weight and horizontal tolerance respectively. Now in the proposed project, these convergent lines will be used as parents to develop Congenic lines.

15.2 ORIGIN OF THE PROPOSAL / RATIONALE OF THE STUDY:

Existence of extreme variations in topography, altitude, soil type, nature, fertility, rainfall, number of rainy days, temperature, humidity etc. in tropical country like India specially Eastern and North Eastern region is a great problem to conduct successful rearing of productive breeds / hybrids. As for example, in West Bengal, silkworm rearing for commercial purpose is practiced five times in a year at farms and farmer's level due to availability of huge mulberry leaves for high rainfall and fertility of soil. But, the practice of bivoltine rearing or productive silkworm breeds or their hybrids is very restricted for highly fluctuating climatic situation consider as an unfavourable (May to September) of this region. So, P1 bivoltine rearing to prepare multi x bi hybrid dfls for three commercial crop (June - July, August - September and November -December) is not successful as fall under unfavourable season and not congenial for bivoltine rearing due to high temperature (>35⁰ C) and high humidity (>85-99 %). Therefore, farmers are forced to restrict their rearing only with Nistari and M12W both as P1 and only Nistari or Nistari x M12W as a commercial hybrid. The P1 bivoltine as a parental rearing (December and February) to prepare multi x bi

hybrid or bi x bi hybrid for two commercial rearing viz., February – March (Falguni) and March – April (Baishakhi) fall under congenial environment and have no problem except low temperature in December. On the other hand, specially P1 bivoltine rearing for preparing multi x bi or bi x bi hybrid in September for Autumn (November) commercial crop falls under most adverse climatic condition though the commercial season in November is the best congenial season for silkworm rearing which in turn results the scarcity of multi x bi and bi x bi commercial dfls to produce good quality and quantity of silk.

It was observed that survival of bivoltine and quality in multivoltine breeds could be increased through Congenic breeding approach during adverse climatic condition. Successfully congenic breeds B.Con.1, B. Con. 4 as high survival bivoltine and M.Con.1 and M.Con.4 as multivoltine congenic breeds for high survival with high cocoon shell weight has been developed as P₁, which is not possible through conventional breeding.

Rapid change in the global climatic situation, to bear this variable changes and to get more success in silk production, it is crucial part of research to bring **Genetic Plasticity in the Gene pool of silkworm**. Thereafter, Congenic Breeding approach will be adopted for introgression a target trait in multivoltine for high cocoon shell weight and the trait horizontal resistant in bivoltine. From the proposed project it is expected that there is fair chance to get genetic plasticity in the gene pool for proposed target trait in the developed breeds.

15.3 RELEVANCE TO THE CURRENT ISSUES & EXPECTED OUTCOME:

Though developed Congenic breeds has provided better performance as a P1 during adverse climatic situation, rapid changes in the Global climatic situation there is a need to develop silkworm breeds to withstand the rapid variation in the climate. So, it is crucial part of research to bring **Genetic Plasticity in the Gene pool of silkworm**. Therefore it is proposed to try further to adopt another breeding approach to assimilate a number of favourable genes for a trait to bring genetic plasticity in the gene pool through aggregating the gene(s) from well-adapted different strains from different geographical zone of India and selection under adverse climate. So there is a chance to bring capacity for adaptation caused by genetic changes *i e.*, **Genetic Plasticity**.

It is expected that there is a fair change to develop silkworm breed having Genetic Plasticity in the gene pool of bivoltine for high cocoon shell weight with higher survival after introgression of this trait from multivoltine breed. Similarly, there is a possibility to develop a multivoltine breed having Genetic Plasticity in the gene pool for horizontal resistant with high cocoon shell weight after introgression of this trait from bivoltine breed.

As congenic breed provides a root of quickly identifying marker linked to traits, may help to identify diversified region at biochemical and DNA level.

15.4 OBJECTIVE:

- **Screening of silkworm breeds for Converged Gene pool having Genetic Plasticity in tolerance and in high cocoon shell weight – done in earlier project**
- **Development of Congenic multivoltine breed from selected lines for high cocoon shell weight and bivoltine breed for horizontal tolerance.**
- **Identification of biochemical / molecular marker associated with the target traits.**

16: The review should restrict to relevant information which gives an insight into the current issue / problem or clues for solving the problem. Any relevant work on other organisms that can provide cues for solving the present problem should be included.

16.1 INTERNATIONAL STATUS:

Time to time different improved silkworm breeds were developed from hybridization followed by line selection *i.e.*, development of **RIL** by different research Institutes and used in hybridization. So far no attempt has been taken to bring or to increase “**Genetic plasticity**” in gene pool of silkworm by aggregating favourable gene(s) for horizontal resistance or for any quantitative trait like cocoon shell weight.

No information is available on development of Congenic line dealing with multiple genes for a character in any beneficial insects or in silkworm, *Bombyx mori* L in any Sericultural Research Institute except **Central Sericulture Research and training institute, Berhampore, West Bengal**. Use of Near Isogenic line (NIL) in plant system was reported by Waugh and Powell, 1992; Haley, *et al.*, 1994, Martin, *et al.*, 1991, Tanksley and Rick, 1980; Tanksley *et al.*, 1981, 1982 Young *et al.*, 1988; Stam and Zeven, 1981; Zeven *et al.*, 1983; Torigoe *et al.*, 1987 for yield improvement, disease resistant and or qualitative improvement. They also suggested that Near Isogenic line (NIL) is suitable for biochemical tools when searching for biochemical or genetic marker/ molecular marker linked to the introgressed locus. Blondon *et al.*, 1994; stated that Near-Isogenic lines (NILs) provides a route for quickly identifying markers linked to a trait of interest. It was also stated that NIL would provide discrete difference in the presence or absence of a target sequence and a small region of flanking DNA. Melchinger, 1996; Michelmore *et al.*, 1991, identified molecular markers linked for disease resistance genes by bulk segregation analysis in plants. Blondon-Adam *et al.*, 1994 stated that SCAR, RAPD and RFLP markers linked to a dominant gene

(Are) conferring resistance to anthracnose in common bean. Zubay, 1986 introduced BH2 gene from B strain mice to A strain mice through development of Near Isogenic line based on single gene concept. Thus suggesting for studying histo-compatibility and serological test for presence of H2 antigen, thus suggesting a possibility to develop a new variety / strain for flesh and disease resistant animal. Earlier Congenic / Near Isogenic approach was not used in any economical insect like silkworm.

Multiple cross system i.e., **Convergent Crossing** approach (**See. Annex. II**) in silkworm breeding as a useful breeding method for genetic assimilation as major traits of silkworm are controlled by multiple genes (Silkworm Breeding,1993, United Nations publication) though its' utility was very scanty in Indian sericulture. So far, no breeding strategy has been adopted to use converged gene pool to bring the genetic plasticity in the developed silkworm breed.

16.2 NATIONAL STATUS:

Last 3-4 decades, Central Sericulture Research and Training Institute under CSB and few University developed a number of improved multivoltine (V_3) i.e., non-hibernating and bivoltine (V_2) i.e., hibernating silkworm breeds following the methods of Recombinant Inbred Line (RIL) development. Developed RIL were used to raise different combinations of hybrid to utilize their hybrid vigour for commercial benefit. A number of workers Sengupta *et al.*, 1971; Sengupta and Datta, 1973; Subba Rao *et al.*, 1990; Roy *et al.*, 1997; 1998 had tried to screen the hybrid (s) for better heterosis having sustainability to various climatic situation at farmers level but the results had no persistency. Till, screening of multi x multi and multi x bi hybrids on seasonal basis (Rao *et al.*, 2004; Singh *et al.*, 2004; Rao *et al.*, 2002 a, b; Rao *et al.*, 2004; Babu *et al.*, 2001; Kumaresan *et al.*, 2003; Vidyunmala *et al.*, 1998; Singh *et al.*, 2001) are the regular breeding approach in Sericultural research. Last few years, study on genetic diversity and other molecular approach in silkworm, *Bombyx mori* by Nagaraju and Goldsmith (2002) and genetic informative ness offered by micro satellite in silkworm genome (Reddy *et al.*, 1999; Nagaraju *et al.*, 2001) among different strains / breeds are well defined but no definite association was established with tolerance and or quantitative trait like cocoon shell weight in silkworm, *Bombyx mori* L.

Generally, rearing of bivoltine (V_2) as a P1 (parental) is a major impediment for their poor survivals during adverse climatic situation (April to September), resulting failure in preparation of hybrid layings. Considering this impediment, at present a few congenic breeds of multivoltine (V_3) for high cocoon shell weight (a multigenic trait) and congenic bivoltine

(V₂) breeds for high survival (multiple genes controlled) were developed using congenic line (Con. L) development breeding approach adopted for multiple genes introgression (Chattopadhyay et al., 2001 a, b; **Annexure -VII**). Following this breeding approach survival character from multivoltine (V₃) CB5 breed was introgressed to JPN bivoltine (V₂) breed by developing CB5 Con.Ow (B.Con.1 - off white cocoon colour), CB5 Con.C (B. Con. 2- golden yellow cocoon colour), CB5 Con.Gc (B. Con.3 -light greenish cocoon colour). Similarly, D6p Con.Ow (B. Con. 4 -off white cocoon colour) was developed using another multivoltine M6DPC as a donor. Nistari was also used as a donor for high survival to develop D6p Con.+c (B. Con. 5 - white cocoon colour) and D6 p Con. F (B. Con. 6 - Flesh colour). All developed congenic bivoltine (V₂) breeds are giving better survival in comparison to traditional any bivoltine (V₂) breed like NB4D2 or other bivoltine breeds where the survival is very meager especially in adverse climatic condition. On the other hand high cocoon shell weight character from (V₂) JPN breed was introgressed to (V₃) CB5 by developing high cocoon shell weight V₃ congenic breeds like CB5 Con.Ow (M.Con.1 -off white cocoon colour), CB5 Con.C (M. Con. 2 -golden yellow cocoon colour); CB5 Con,Gc (M. Con.3 - light green cocoon colour) while high cocoon shell weight of D6p was introgressed in M6DPC i.e., M6DPC Con.C (M. Con. 4 - golden yellow cocoon colour). One Nistari based RBL3 multivoltine line as RBL3+p F (flesh colour) was also developed (congenic stage is in progress). All congenic multivoltine (V₃) breeds are given high cocoon shell weight ranges from 0.255 to 0.260g with improvement on other yield contributing parameters and survival (< Nistari) than any improved multivoltine (V₃) breeds having cocoon shell weight 0.115g to 0.135g (> Nistari). Developed (V₂) breeds B.Con.1, B. Con.4 and (V₃) M.Con.1, M.Con.4 are placed under farms level trial for testing the survival and improvement on cocoon shell weight of bivoltine and multivoltine breeds respectively during different seasons of P1 rearing and their hybrids i.e., congenic multi x bi hybrid viz., M.Con.1 x B.Con.4; M.Con.4 x B.Con.4 and congenic bi x bi hybrid viz., B.Con.1 x B.Con.4 and its reciprocal at farmers level in different districts of West Bengal through a collaborative project with DOS, West Bengal. At the same time multi x bi congenic hybrids viz. M.Con.1 x B.Con.4, M.Con.4 x B.Con.4 and Multi x Multi viz. M.Con.1 x M.Con.4, N+p x M.Con.4 and its reciprocal were placed under Mulberry Silkworm hybrid Authorization Programmed (MSRAP-VII) at national level. The result depicted that all the hybrids performed better in different test center. Prior to that, performances of congenic multi x multi and congenic bi x bi hybrids were tested for one year at different REC center under CSR&TI. The result depicted that congenic hybrids are performing better by obtaining first rank against the control N x NB4D2. Verma *et al.*, (2003, 2005) also observed that congenic hybrid of V₃ x V₃ or V₃ x V₂ and V₃ Congenic breed x V₂ syngenic line (Syn.L) give heterotic and heterobeltiotic-genetic interaction respectively on

single cocoon shell weight and total yield along with improvement in other economic characters.

During the course of development of syngenic lines, one thermo-stable ($80\pm 1^\circ\text{C}$) β -esterase band was identified in haemolymph, which is naturally present in one of the V_3 breeds. This thermo stable esterase possessor native protein was considered as one of the biochemical factors for thermo-tolerant (Chattopadhyay *et al.*, 2001c). It was also identified that apparent native protein of 224 kDa in haemolymph of multivoltine (V_3) breeds at pH. 8.3 is α -esterase possessor and associated with survival when introgressed to congenic bivoltine (V_2) breed for high survival. Another 180 kDa native protein in haemolymph of bivoltine breeds at pH. 8.3 was also identified as α -esterase possessor and associated with high cocoon shell weight when introgression to congenic multivoltine (V_3) for high cocoon shell weight (Chattopadhyay *et al.*, 2005a,b; see; Final Technical report of **CSIR** funded project, scheme No. 37(1095) / 02 / EMR-II, 2005 and **CSB** funded project No.AIG-002). Isozyme possessor apparent native protein pattern of Amylase, Esterase, Acid phosphatase and Malate dehydrogenase were used to draw schematic native proteins zymogram depicted that few native proteins are directly associated with non-hibernation and hibernation character by using developed multivoltine and bivoltine congenic breeds of silkworm, *Bombyx mori*, L. Chattopadhyay *et al.*, 2004,2005 (CSB & CSIR final report) also supported the views that Congenic Breed like Near Isogenic Line (NIL) is suitable for biochemical tools when searching for biochemical marker linked to the introgressed locus. So far, no breeding strategy has been proposed to develop new breed using converged gene pool to bring the genetic plasticity in the breed.

16.3 IMPORTANCE OF THE PROPOSED PROJECT IN THE CONTEXT OF CURRENT STATUS:

It is a challenge to overcome the problem by raising a sustainable bivoltine breeds with good survival and multivoltine breeds with high shell weight for adverse seasons (April, June and September). The developed Congenic breeds have come out to solve such problem so far now under popularization stage. Besides, **Rapid change in the global climatic situation, to bear this variable changes** sustainable breeds having genetic plasticity in gene pool are urgently required to increase the production of P1 bivoltine cocoon and quality multivoltine cocoon. This proposed project might help to develop bivoltine and multivoltine breeds to combat the present and forth coming climatic problems to raise multi x bi hybrid dfls through by which the sericulture farmers will get more financial benefit. In addition, introduction of bivoltine rearing at farmers level through out the year will be possible to boost the mulberry raw silk production under 11th year plan.

**16.4 ANTICIPATED PRODUCTS, PROCESSES/ TECHNOLOGY PACKAGE
INFORMATION OR OTHER OUTCOME FROM THE PROJECT AND THEIR
EXPECTED UTILITY:**

Developed breeds to be exploited as a P1 breeds at farms and farmers level to overcome the scarcity of multi x bi or bi x bi dfls during commercial seasons.

**16.5 EXPERTISE AVAILABLE WITH PROPOSED INVESTIGATION GROUP /
INSTITUTION
ON THE SUBJECT OF THE PROJECT:**

Principal Investigator is experienced to operate the total project work and Co-Investigator is capable to carry out the work associated with biochemical part.

16.6 LIST OF FIVE EXPERTS IN INDIA IN THE PROPOSED SUBJECT AREA

Sl. No.	Name	Designation	Address
1	Prof. R. N. Chatterjee	Professor, Department of Zoology	Genetic Research Unit, Dept.of Zoology, University of Calcutta, 35 Ballygunj Circular Road, Kolkata-700019
2	Prof. P. K. Das	Professor, Department of Plant Breeding & Genetics,	B.C.K.Viswa Vidyalya, Mohanpur, Nadia W.B.
3	Prof. V. Srinivas Reddy	Professor, Department of Sericulture Science	Dept. of Study in Sericulture Science, University of Mysore, Manasagangothri, Musore-570006
4	Prof. M. Krishnan	Professor, Department of Biotechnology	Bharati Darsan University, Trichy, T.N.
5	Prof. J.P.N. Pathak	Professor, Department of Zoology	Ujjain University, M.P.

17. WORK PLAN

17.1. Methodology:

Collection of Collection of Six Bivoltine and Six Multivoltine Breeds/ Strains from different geographical zones of India (**Annexure – I**) and convention silkworm rearing method will be followed for assessment of quantitative and qualitative characters in West Bengal climatic condition with molecular characterization. Thereafter convergent breeding approach will be adopted to make broad genetic base to bring genetic plasticity in bivoltine for high cocoon shell weight and multivoltine for high survival- pupation percentage (**Annexure-IV**) with molecular characterization. Identified lines will be used to develop congenic productive and tolerant congenic silkworm breed with molecular characterization. The molecular characterization will be done as per stander method used internationally.

17.2. Organization of Work Elements

Name	Work to be done
Dr. Kanika Trivedy	Over all coordination
Dr. A. K. Verma Principal Investigator	E01, and E02 and Molecular characterization, compilation and interpretation of data, submission of report time to time and final report preparation.
Sri N. Chandrakant Co-Investigator-1	Associated with E01, and Molecular characterization, data compilation and to assist in report and final report preparation
Mr.N.B.Kar	Associated with E01, E02 and E03 for post cocoon Analysis

THE EXPERIMENTS ASSOCIATED WITH THIS PROJECT ARE AS FOLLOWS:

EXPERIMENT No. : **E01**
 EXPERIMENT TITLE : **Development of productive and tolerant Congenic Breeds and Molecular Characterization.**
 OBJECTIVES : Development of productive and tolerant Congenic Silkworm Breeds with molecular characterization.
 STARTING DATE : After completion of E02.
 COMPLETING DATE : 36 months from the development of converge gene pool
 METHODOLOGY Congenic breeding approach (Chattopadhyay et al 2001a, b., 2005 (**See Annexure-III**)) followed by standard for Molecular Characterization

ACTIVITY	TIME EXPECTED	
	DATE OF STARTING	DATE OF CLOSING
Development of Congenic Bivoltine and Multivoltine breeds	After completion of E02	24 months after the completion of E02

EXPERIMENT No. : **E02**
 EXPERIMENT TITLE : **Identification of biochemical / molecular marker associated with the target traits.**

OBJECTIVES : To identify biochemical / molecular marker associated with high

shell character of developed multivoltine congenic line and high survival of developed bivoltine congenic lines

STARTING DATE : After completion of 24 months of E01.

COMPLETING DATE : 12 months

METHODOLOGY **Congenic breeding approach (Chattopadhyay et al 2001a, b., 2005 (See Annexure-III) followed by standard for Molecular Characterization**

ACTIVITY	TIME EXPECTED	
	DATE OF STARTING	DATE OF CLOSING
To identify biochemical / molecular marker associated with target characters	After 24 months of E02	12 months from start

17.3. Proprietary/ Patented items, if any, expected to be used for this Project:

From earlier project work, two developed Congenic breeds from viz., B.Con.4 and M.Con.4 will be used as breeding materials.

17.4. Suggested plan of action for utilization of the expected outcome from the project:

It is expected that developed breeds will be utilized directly for commercial exploitation with out further hybridization or may be used as a P1 for commercial hybridization if it shows heterotic affect.

17.5. TIME SCHEDULE OF ACTIVITIES GIVING MILESTONES

Sl. No.	Milestone/ Activity	Expected Date of		Expected Outcome /visible/ measurable indicator
		Starting	Completion	
E01.	Development of productive and tolerant Congenic breeds and Molecular Characterization.	After the development of converge gene pool of multi and bivoltine lines with target characters	36 months after the start See: Annexure III	Multivoltine Congenic breed with high shell weight and congenic bivoltine breed with high pupation rate and molecular characterization.

E02.	Identification of biochemical / molecular marker associated with the target traits.	After 24 months	12 months after the start See: Annexure III	Biochemical/ Molecular (s) if any, associated with the target characters
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PART IV: BUDGET PARTICULARS:

18. BUDGET (in Lakhs):

C. BUDGET ESTIMATES: SUMMARY

ITEM					
BUDGET					
	1 st Year	2 nd Year	3 rd Year		Total
A. Recurring					
1. Remuneration /Salaries	-	-	-		-
2. Consumables(including chemicals)	0.20	0.50	0.50		1.20
3. Travel	-	0.20	0.20		0.40
4. Other Costs	0.10	0.10	0.10		0.30
B. Non-recurring					
A.C.-1, Microscope-1 & Cocoon weighing Machine-1	1.5	-	-		1.50
Grand Total (A + B)	1.80	0.80	0.80		3.40

DETAIL BUDGET FOR REMUNERATIONS / SARARIES(In Lakhs)

Designation Number of Persons	1 st year	2 nd year	3 rd year		Total
	-	-	-		-
Part time	-	-	-		-
Grand Total	-	-	-		-

DETAIL BUDGET FOR TRAVEL:

ITEM BUDGET	1 st Year	2 nd Year	3 rd Year		Total
Travel 1. Local	-	0.05	0.05		0.10
2. Out Station	-	0.15	0.15		0.30
TOTAL	-	0.20	0.20		0.40

DETAIL BUDGET FOR OTHER COSTS:

ITEM BUDGET	1 st Year	2 nd Year	3 rd Year		Total
a) Contingencies	0.10	0.10	0.10		0.30
b) Others	0.10	0.10	0.10		0.30
Total	0.20	0.20	0.20		0.60

PART VI: REFERENCES

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Annexure-I

The following breeds have been considered for the proposed Project entitled “Development of Silkworm (*Bombyx mori* L) Breeds from a Gene pool with Higher Genetic Plasticity.”

Bivoltine (V₂)

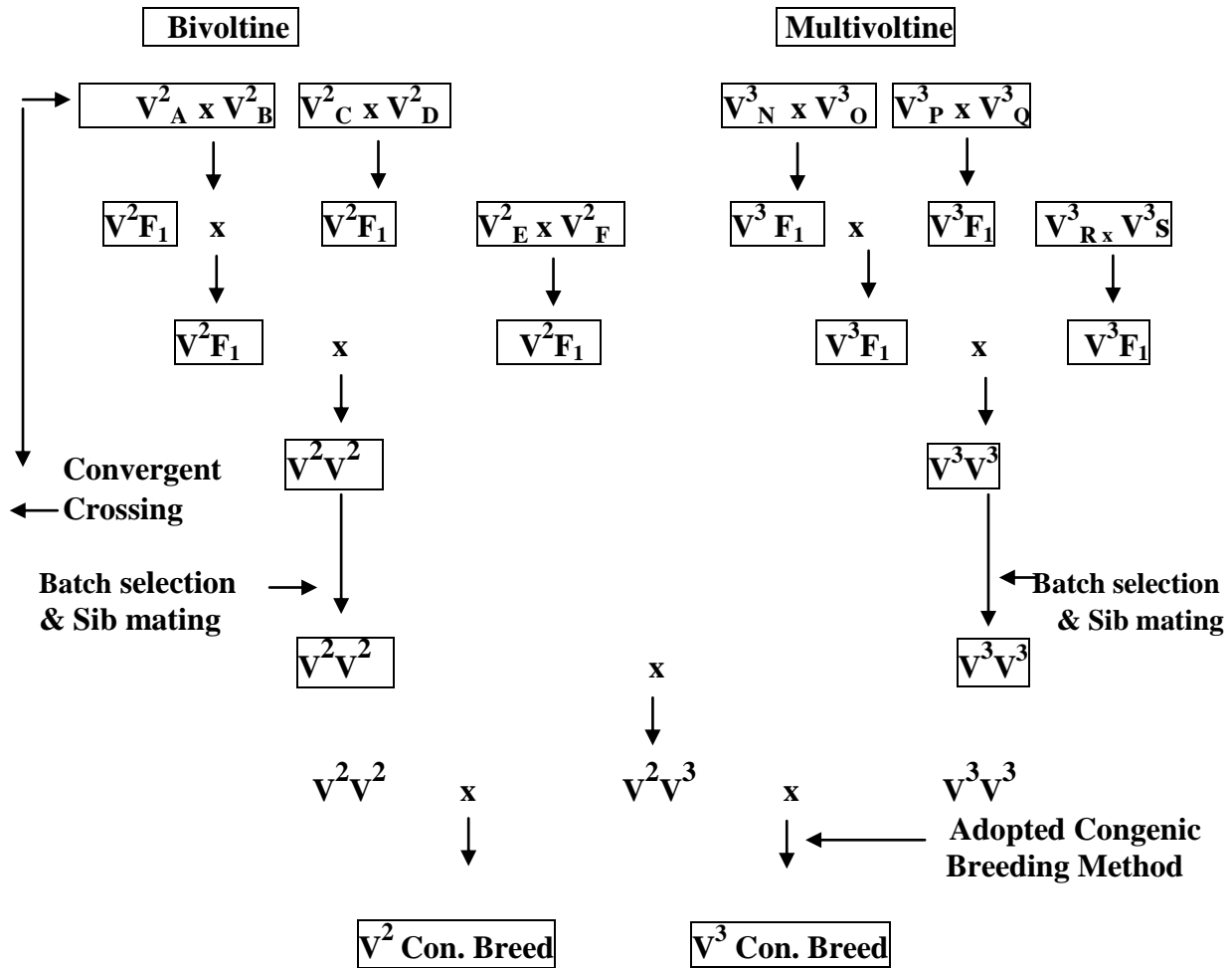
Sl. No	Breed's Name	Origin	Cocoon shape	Cocoon Colour	Shell Wt.	Shell %	Pupae%
1	B.Con-4	CSR&TI, Berhampore (Variable climate)	Oval slightly Cons.	Off white	0.318	18.06	Un.F- 50.8 Fav-73.0
2.	KPG-A	RSRS Kalimpong (Congenial climate)	Oval	White	0.275	21.90	85.70
3.	RSJ-14	RSRS Jammu (Congenial climate)	Oval	Off white	0.463	24.11	96.00
4	DUN-21	RSRS, Sahaspur (Low tempt & humidity)	Constricted	Off white	0.450	23.00	95.00
5	CSR-2	CSR&TI, Mysore (Congenial climate)	Oval	White	0.360	22.15	86.40
6	APS- 45	APSSRDI, Hindupur (Variable climate)	Oval	White	0.32	19.43	92.58

Multivoltine (V₃)

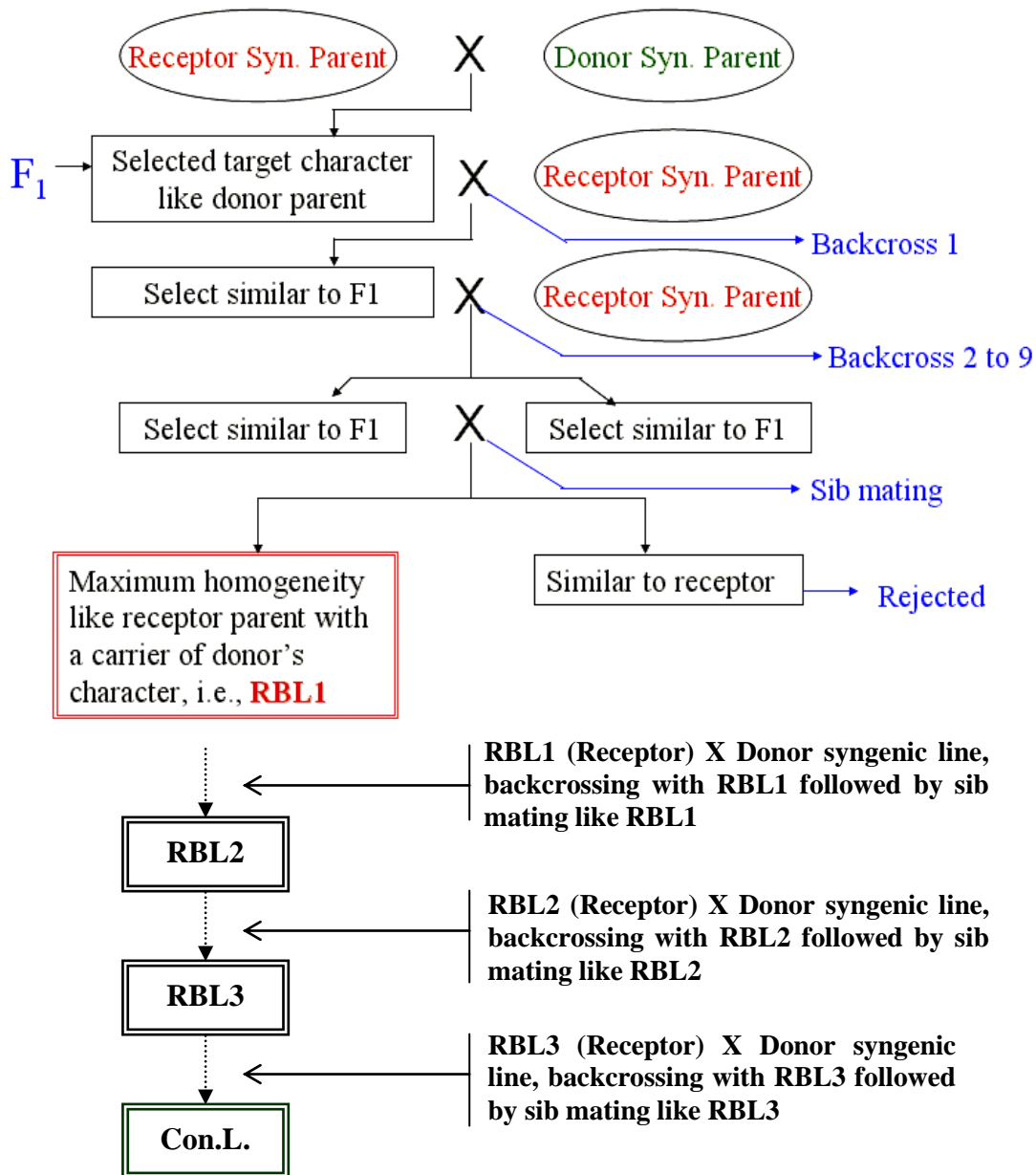
Sl. No.	Breed's Name	Origin	Cocoon shape	Cocoon Colour	Shell Wt.	Shell %	Pupae %
1	M.Con4	CSR&TI, Berhampore (Variable climate)	Oval	Yellow	0.232	17.03	UnF-59.45 Fev.-75.0
2.	Nistari	West Bengal (Variable climate)	Spindle	Yellow	0.114	12.13	UnF-65.58 Fev-76.01
3.	APM-1	APSSRDI, Andra Pradesh	Spindle	Greenish Yellow	0.170	14.85	88.0
4	Cambodge	Exotic & Viral resistant	Spindle	Yellow	0.140	14.20	91.0
5	Pure Mysore	CSR&TI, Mysore (Congenial climate)	Spindle	Light greenish	0.140	14.07	83.0
6	C'nichi	Exotic, Maintained at CSR&TI, Mysore	Constricted	white	0.12	11.99	84.0

Annexure-II

Scheme for Development of a Line / Breed having Genetic Plasticity



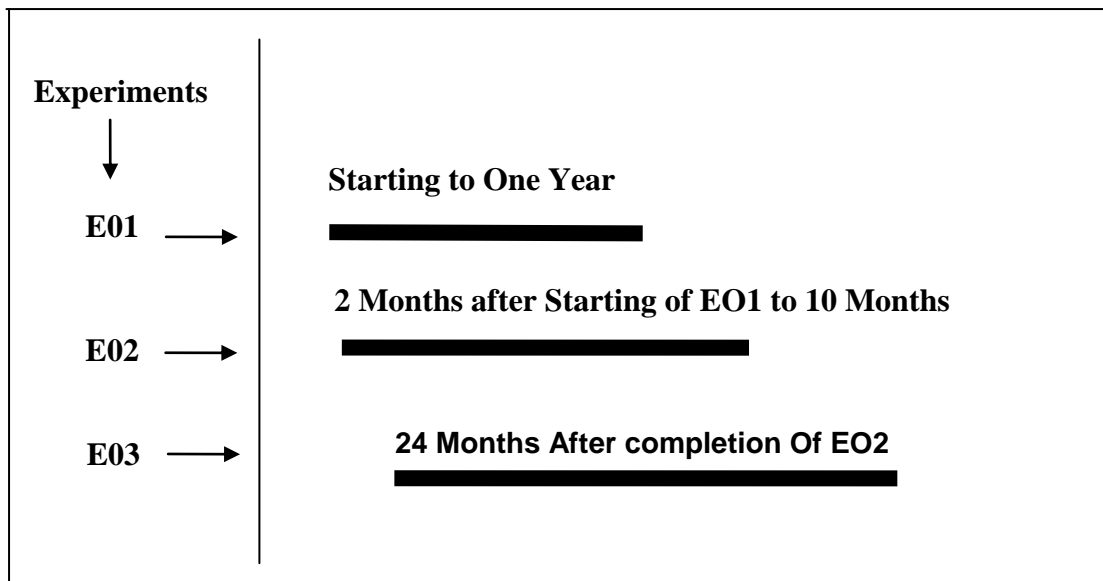
**Scheme for Development of Congenic Breed
(Dealing with Multigenic trait)**



* Number of crossing with donor depends upon the transgression of target trait

- Chattopadhyay, G. K. *et al.*, 2001 a, b.

ANNEXURE-IV



E01: Assessment of silkworm breeds / strains and Molecular characterization.

E02: Development of Multivoltine and bivoltine lines from converged gene pool and Molecular characterization

E03: Development of productive and tolerant congenic breeds and Molecular Characterization.

FLOW CHART

Collection of Six Bivoltine and Six Multivoltine Breeds/ Strains from Different geographical zones of India



Characterization of Different breeds Qualitatively, Quantitatively, and Bio-chemically and at Molecular level



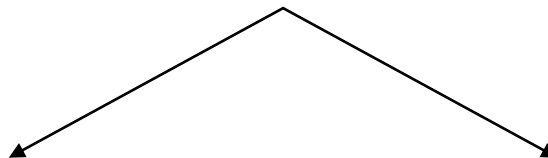
Raising of converge Gene pool to bring genetic plasticity both in Bivoltine and Multivoltine for high cocoon shell weight and high survival (pupation) respectively



Selection of Bivoltine line with high cocoon shell weight and Multivoltine line with high survival



Development of Bivoltine congenic breed with High survival and multivoltine breed with high cocoon shell weight.



PCR based

Isozyme

Dated: 22.12.2010.

To,
The Director,
C S R & T I
Berhampore

Sir,

Sub: Submission of New Proposed Project Reg:

I am submitting the proposed Project entitled “**Development of Silkworm (*Bombyx mori* L.) Breeds form a Gene pool with higher Genetic Plasticity**” as per the discussion of last RC.

This is for your kind information and needful action.

With regards,

Yours faithfully,

(Dr. G.K.Chattopadhyay)
Scientist –C
SBG, CSR&TI. Berhampore

PART VII: BIODATA OF PRINCIPAL INVESTIGATOR

1. Full Name (In Block letters): **DR. GAUTAM KUMAR CHATTOPADHYAY**
2. Designation:, Scientist –‘C’
3. Department / Institute: Central Sericultural Research & Training
University. Institute, Berhampore, W.B
4. Date of birth: 27th October 1954
5. Sex: Male
6. Education (Post Graduation onwards & Professional Career)

Name of the University	Degree Passed	Year of Passing	Subjects taken with Specialization
Burdwan	B.Sc (Hons).	1974	Zoology (Hons), Botany, Chemistry
Kalyani	M.Sc	1976	Zoology (Cytogenetics special)
-DO-	Ph.D.	1987	Zoology. Subject covered- Cytogenetics, Chromosomal mutation, Embryology, Reproductive biology, Histology & Teratology.

7. AWARDS: Nil

TRAINING UNDERGONE BY PI

COURSE NAME/ SUBJECT	ADDRESS OF INSTITUTE	SPONSORING AGENCY
1. DBT laboratory Workshop entitled "Cloning and characterization of animal genes".	Bose Institute, Kolkata.	CSB
2. "Detection of environmental mutagens & mutagenesis at DNA level"	BARC, Mumbai and Calcutta University	Kalyani University, Kalyani
3. Workshop on Bioinformatics in Genomic & Proteomics. 721302. West Bengal.	Department of Biotechnology, Indian Institute of Technology, Kharagpur. W.B	CSB

8. POSITIONS HELD/ RESEARCH EXPERIENCE IN VARIOUS INSTITUTES: **NA**

9. MEMBERHIPS / FELLOWSHIPS:

10. PATENTS: NIA

11. PUBLICATIONS:

Books - Nil

Research Paper, Report: 20 papers published in National & international Journals
and 22 Abstracts in conference and seminars

General Articles: Nil

LIST OF IMPORTANT PUBLICATIONS WHOSE CONTENTS CAN BE USED IN THE
PROPOSED AREA OF WORK:

Chattopadhyay, G. K., Sengupta, A. K., Verma, A. K., Sen, S. K. and Saratchandra, B. (2001a) Utilization of congenic line in silkworm breeding. In: *Perspectives in Cytology & Genetics*. G.K. Manna and Roy, S.C (eds.) **10: 717-724.**

Chattopadhyay, G. K., Sengupta, A. K., Verma, A. K., Sen, S .K. and Saratchandra, B. (2001b) Transgression of shell weight - A multigenic trait, through development of congenic breed in tropical silkworm *Bombyx mori* L. *Sericologia*. **41(1): 33-42.**

Chattopadhyay, G. K., Sengupta, A. K., Verma, A. K., Sen, S. K. and Saratchandra, B. (2001 c) Esterase isozyme polymorphism, Specific and nonspecific esterase, syngenic line development and natural occurrence of a thermo-stable esterase in tropical silkworm *Bombyx mori* L., *Insect Biochem. Mol. Biol.* **31: 1191-1199.**

Chattopadhyay, G. K., Verma, A. K., Sengupta, M., Sengupta, A. K., Das, S. K and Raje Urs, S. (2004) α -and β -amylase isozyme possessor native proteins in tropical silkworm *Bombyx mori* L. *Int. J. Indust. Entomol.* **8(2): 21-27.**

Chattopadhyay, G. K.; Verma, A. K.; Das, S. K. and Sarkar, A.(2005a) Acid phosphatase isozyme possessor native proteins pattern in Congenic breeds of silkworm, *Bombyx mori* L. In: *12th All India Congress of Cytology and genetics. Institute of Science, Mumbai .C-38.*

Chattopadhyay, G. K.; Verma, A.K., Das, S. K. and Sarkar. A. (2005b) Esterase-a Biochemical marker for quantitative traits of silkworm, *Bombyx mori* L. In: *National Symposium on Development Dynamics. Indian Society of Developmental Biologists (Nov.23rd -25th)*. Department of Zoology. University of Kalyani, Kalyani-741235. West Bengal.

12. PROJECT (S) SUBMITTED/ BEING PERSUED / CARRIED OUT BY INVESTIGATOR

Title of the project	Funding Agency	Duration From To	No. of Scientists / Associates	Project Cost (Rs.InLakh)
1. Restriction filament length polymorphism studies in Silkworm, <i>Bombyx mori</i> L. Co-Investigator	DBT, Delhi	1990– 93 Concluded	2	40.00
2. Utilization of syngenic lines for improvement of shell weight and survival in silkworm, <i>Bombyx mori</i> L, Principal Investigator	Central Silk Board, Bangalore	Dec-1998 to Mar, 2004 Concluded	3	19.30
3. Introgression of high shell weight, high survival character(s) / gene(s) through the development of multivoltine and bivoltine congenic breeds and identification of biochemical markers in silkworm, <i>Bombyx mori</i> L. Principal Investigator	CSIR, Delhi	April, 2002 to March, 2005 Concluded	2	6.00
4. On farm trial of congenic silkworm hybrids for commercial exploitation. Principal Investigator	Central Silk Board, Bangalore	Sept, 2004 to Dec' 2005 Concluded	4	1.00
5. On farm trial of evolved bivoltine and multivoltine Congenic breeds and their hybrid performance at farmer's level (In collaboration with DOS, W.B Principal Investigator	Central Silk Board, Bangalore	Dec' 05 - Nov' 2007 Concluded	10	11.30

13. HIGHLIGHTS OF OUTCOME / PROGRESS OF THE PROJECT (S) HANDLED DURING THE PAST 10 YEARS, THEIR OUTCOME AND UTILIZATION (IN 200 WORDS):

Developed a **method for introgression of a trait controlled by multiple genes** for developing Congenic Breed (Chattopadhyay et al., 2001a, b 2005).

Developed **four promising silkworm breeds** viz., M Con.1, M Con.4 (Multivoltine), B Con.1, B. Con.4 (Bivoltine)

Identified **five promising hybrid** combinations.

M Con.1 x M Con.4, N x M Con.4 (Multivoltine x Multivoltine)

M Con.1 x B Con.4, M Con.4 x B Con.4 (Multivoltine x Bivoltine)

B Con. 1 x B Con.4 (Bivoltine x Bivoltine)

Registration of breeds: Six (6) congenic breeds viz., V³ CB5-Con.Ow, V³ M6DPC-Con.C, V² D6p-Con.Ow, V² D6p- Conc., V² D6p-Con.F and one sex limited breed (JPN ^{+HS}) was send for registration at CSGRC, Hosur.

Biochemical study / Markers: Established that **amylase** is one of the most important enzymes in tropical silkworm having **positive correlation with high survival**.

Identified **biochemical marker for high survival (224kDa at pH-8.5)**. The apparent native protein in haemolymph is the possessor of α -Est s are exclusively present in multivoltine.

Identified **biochemical marker for high cocoon shell weight (180 kDa at pH-8.5)**. The apparent native protein in haemolymph is the possessor of α -Est s and exclusively present in bivoltine.

Identified the existence of **β amylase** in haemolymph and digestive of Silkworm, *Bombyx mori* L.

Identified specific and non-specific esterases using α - and β -naphthyl-acetate separately as non-specific substrates. The non-specific β -esterase-Est-3 in haemolymph is a **thermo-stable enzyme ($80 \pm 1^\circ\text{C}$), which could be considered as one of the molecular factor for thermo-tolerance**.

Identified some **Isozyme possessor native proteins** are associated with **non-hibernation and hibernation character** of silkworm. (CSIR Final report)

LIST OF IMPORTANT PUBLICATIONS

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- 2 Sarkar, C. S. and **Chattopadhyay, G. K.** (1984) Detection of C- band in bone marrow chromosome of mice, *Mus musculus* by an acetic saline –Giemsa (ASG) technique. In: *Perspectives in Cytology & Genetics*. Ed by G. K. Manna and U. Sinha. **4**: 411-414.
- 3 **Chattopadhyay, G. K.** (1984) Effect of bacterium, *Pseudomonas aeruginosa* on the sperm head morphology of mice, *Mus musculus*. In: *Perspectives in Cytology & Genetics*. Ed by G. K. Manna and U. Sinha, **5**: 625-628.
- 4 Sahu, C. R, **Chattopadhyay, G .K.** and Bose, A. K. (1984) Effect of bacterium, *Pseudomonas aeruginosa* on the liver and kidney mice. In: *Perspectives in Cytology & Genetics*. Ed by G. K. Manna and U. Sinha , **5**: 687-690.
- 5 Manna, G. K. and **Chattopadhyay, G. K.** (1987) Precocious centromeric separation in five groups in control and *Pseudomonas aeruginosa* culture treated mice and their progeny. **Current Science**, **15**: 759-769.
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- 8 **Chattopadhyay, G.K.**, Sengupta, A. K.; Verma, A. K, Sen, S. K. and Saratchandra, B. (2001c) Esterase isozyme polymorphism, Specific and nonspecific esterase, syngenic line development and natural occurrence of a thermostable esterase in tropical silkworm *Bombyx mori* L., **Insect Biochem. Mol. Biol. (USA)**, **31**: 1191-1199.
- 9 **Chattopadhyay, G.K.**, Sengupta, A. K.; Verma, A. K, Sen, S. K. and Saratchandra, B. (2000a)Utilization of congenic line in silkworm breeding. In: *Pers.in Cytol. & Genet*. Ed by G. K. Manna and S. Roy. **10**: 717-724.
- 10 Manna, G.K, **Chattopadhyay, G. K.** and Dey, Mira. (2001) Genotoxic, mutagenic and teratogenic potentials of the bacterium, *Pseudimonas aeruginosa* tested in experimentally treated albino mice and its follow up in F1 and F2 generations. In: *Per. in Cyto & Gene*. Ed by G. K. Manna and S. Roy. **10**: 865-890.
- 11 Verma, A. K, **Chattopadhyay, G. K.**; Sengupta. M, Sengupta, A. K. Das, S. K. and RajeUrs, S. (2003) Expression of heterotic genetic interaction among

multivoltine backcross / congenic line for higher shell weight of silkworm *Bombyx mori* L. *Inter.J. of Indust. Ent. (IJIE), Korea. 7(1): 69-73.*

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- 13 Verma, A. K, **Chattopadhyay, G. K.**; Sengupta, M.; Sengupta, A. K.; Das, S.K. and Raje Urs, S. (2005) Heterobeltiotic genetic interaction between congenic and syngenic breeds of silkworm *Bombyx mori* L. *Inter.J. of Indust. Ent. (IJIE), Korea. 11(2): 119-124*
- 14 Das, S. K, Moorthy, S. M.; **Chattopadhyay, G. K.**, Verma, A. K, Ghosh, B, Rao, P. R. T, Mukherjee, S, Sengupta, A. K. and Sarkar. A (2005) Breeding strategies for high humidity and high temperature conditions of Eastern region. In: Mulberry silkworm Breeders Meet. Central Sericultural Research & Training Institute. Berhamporew-742101, West Bengal: **42-48.**
- 15 S.K. Das, S.M.Moorthy, **G.K. Chattopadhyay**, A.K. Verma, B.Ghosh, P.R.T. Rao, A.K. Sengupta and A.Sarkar (2006). Silkworm Breeds and Hybrids for Eastern India (Abstract). **Workshop** on Appropriate technology for Mulberry Sericulture In Eastern and N-E India, Berhampore, West Bengal, 17th-18th, January, **91-96.**
- 16 Verma, A. K, **Chattopadhyay, G. K.**; Sengupta, A. K, Das, S. K. and Sarkar. A. (2006) New Multi x Bi silkworm hybrids for Eastern India. In: *Workshop* on appropriate technology for Mulberry sericulture in Eastern and North Eastern India. 17th -18th January. Central Sericultural Research & Training Institute. Berhampore-742101, West Bengal: **97-100**
- 17 Das, S.K, **Chattopadhyay, G.K.**, Verma, A.K., Sengupta, A.K and Sarkar,A. 2005. Development of High yielding Silkworm Breeds of *Bombyx mori* L. for Eastern India through Congenic line breeding approach. In: “**20th Cong.of intern.Seri. commission**”, **Bangalore. Vol .I, 268- 272.**
- 18 Das, S. K, Moorthy, S. M.; **Chattopadhyay, G. K.**; Mondal, K. and Bajpai, A. K (2008) Development of silkworm breeds and hybrids for the plains of eastern India. Breeders Meet (2008). Central Sericultural Research & Training Institute. Mysore, pp 6-11
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- 20 Saha, A. K, Suresh kumar, N, **Chattopadhyay,G. K.**, Lakshmi, H, Majumder.M. K and Khatri. R. K (2010) Present status and future strategies for development of silkworm breeds / hybrids for eastern and North- Eastern India. Interactive session on Silkworm Breeding. 2nd and 3rd December at SSBS, Coonoor. pp. 17 – 21.

ABSTRACTS

- 1 Manna, G. K. and **Chattopadhyay, G. K.** (1984) Assessment of somatic and germinal chromosome aberration and the effect of sperm head morphology and genital tissues in F1 and F2 generation of *Pseudomonas aeruginosa* treated male parent mated at the 4th week to virgin females. ***International Symposium of Foetal Biology***. University of Agriculture and Technology. Mathura. pp. 34- 40.
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- 4 Mukherjee, P. K, **Chattopadhyay, G. K.**, Sahu, C. R. and Manna, G. K. (1982) Effect of Endrin on muscle protein of *Talapia mosambica*. *Proceeding of 69th session of All India Science Congress. Section-Zoology Entomology and Fisheries. Part-III*
- 5 **Chattopadhyay, G. K.** and Manna, G. K (1983) Effect of bacterium, *Pseudomonas aeruginosa* on the germinal chromosome and sperm head morphology of male mice *Proceeding of 70th session of All India Science Congress. Section-Zoology Entomology and Fisheries. Part III.*
- 6 **Chattopadhyay, G. K.** and Manna, G. K. (1985) Effect of bacterium, *Pseudomonas aeruginosa* on the germinal chromosome of male mice. *Proceeding of 72th session of All India Science Congress. Section-Zoology Entomology and Fisheries. Part-III.*
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- 9 Chatterjee, S. N, **Chattopadhyay, G. K.** and Naidu, W. D. (1987) Genesis of Amylase enzyme in multivoltine and bivoltine races. *News Letter. C.S.R &T. 2(2).* PP-5.
- 10 Chatterjee, S. N, Abraham, E. G. and **Chattopadhyay, G. K.** (1987) Genetic variability for Amylase enzyme activity. *News Letter. CSR &TI- 2(2).*

- 11 Shivakumar, G. R, Bhargava, S. K. and **Chattopadhyay, G.K.** (1987) Analysis of phenotypic and genotypic variance. *News Letter. CSR &Ti* -**2(3)**.
- 12 Chatterjee, S. N, Abrahman, E. G. and **Chattopadhyay, G. K.** (1987) Digestive enzymes, molecular heterosis Amylase activity in hybrids. *News Letter. CSR &T I*-**2(2)**.
- 13 Chatterjee, S. N, **Chattopadhyay, G. K.**, Rao, G. P.; Nirmalkumar, S. and Sunder, S. (1989) Variability of digestive juice amylase in mulberry silkworm, *News Letter. CSR &Ti.* **3(4)**.
- 14 Chatterjee, S.N, **Chattopadhyay, G. K.** and Naidu, W.D. (1988) Genetic variability of digestive juice amylase in multivoltine and bivoltine races. *News Letter. C.S.R. &T.I*- **3(1)**.
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- 16 **Chattopadhyay, G. K.**, Rao, G. P, Aswath, S. R. and Chatterjee, S. N. (1989) Studies on Protease activity in digestive juice of different silkworm races of *Bombyx mori* L. *News Letter. CSR&TI.* **4(2)**.
- 17 **Chattopadhyay, G. K.**, Verma, A. K, Sengupta, A. K.; Das, S. K. and Raje Urs, S. (2003). α and β amylase isozyme possessor native proteins in tropical silkworm. *Bombyx mori* L. In: National Conference on Tropical Sericulture for Global Competitiveness, 5-7 November. At central Sericultural Research and Training Institute, Mysore.
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- 20 **Chattopadhyay, G. K.**, Verma, A. K, Das, S. K. and Sarkar, A (2005a) Acid phosphatase isozyme possessor native proteins pattern in Congenic breeds of silkworm, *Bombyx mori* L. In: *12th All India Congress of Cytology and genetics.* Institute of Science, Mumbai .C-38.
- 21 **Chattopadhyay, G. K.**, Verma, A.K., Das, S. K. and Sarkar. A. (2005b) Esterase-a biochemical marker for quantitative traits of silkworm, *Bombyx mori* L. In: **National Symposium on Development Dynamics. Indian Society of Developmental Biologists (Nov.23rd -25th).** Department of Zoology. University of Kalyani, Kalyani-741235. West Bengal.
- 22 **Chattopadhyay, G. K.**, Sengupta, A. K, Das, S. K. and Sarkar, A. (2006). Identification and use of marker –a technology to develop productive and stress tolerant silkworm, *Bombyx mori* L. In: Workshop on appropriate technology for Mulberry sericulture in Eastern and North Eastern India. 17th -18th January.

PART VII: BIODATA OF PROJECT CO- INVESTIGATOR :

Name : Dr. H. Lakshmi

Designation : Scientist -B

Department : Silkworm Breeding and Genetics Section

Date of Birth: 10.08.73 Sex: Female

Educational Qualifications:

Sl No.	Degree	Name of Institution	Year	Subjects
1	B.Sc	Sri Krishnadevaraya	1993	Botany, Zoology, Chemistry
2	M.Sc	University	1996	Sericulture Technology
3	Ph.D	CSRTI, Mysore (Mysore University) University of Mysore	2009	Sericulture Title: " Studies on the development of bivoltine hybrids of silkworm <i>Bombyx mori</i> L for tropical conditions of Andhra Pradesh" Guide : Dr. Chandrashekharaiiah, Former Director of APSSRDI

Field of Specialization: Silkworm Breeding

Details of Experience: 13 Years experience as Research Assistant in APSSRDI, Hindupur in Bivoltine Silkworm Breeding Division from 1997 to 2010.

Number of Publications: 34

Research papers: 22 (5 international papers, 7 National Papers, 10 research papers presented in Seminars/conferences)

Popular articles : 11

Book : 01

Seminars Attended: Five

Significant research achievements :

- ❖ I was involved in the development of bivoltine hybrids namely, Hemavathy (APS5 x APS4), Kalpatharuvu (APS9 x APS8), APS45 x APS12, BBO5 x BBP1, SLO1 x SLP2, APSHTO5 x APSHTP2 and APS67 x APS20.

I have been fully involved in the following Research activities in Silkworm, *Bombyx mori* L with particular reference to **SILKWORM BREEDING**

1. Breeding for robust and high yielding bivoltine strains suited to tropics
2. Collection, categorization, utilization and maintenance of silkworm gene bank
3. Evaluation of bivoltine silkworm hybrids
4. Breeding for specific traits viz., longer filament and thin filament size
5. Evolution of viable sex-limited breeds
6. Breeding for high temperature tolerant silkworm breeds
7. Maintenance and multiplication of 47 Basic stocks.
8. Involved in rearing of parental breeds for Race Authorization Test Phase VII and VII (2004 & 2009).

9. Conducted Training Programs to Women Sericulturists and Departmental Staff in silkworm rearing technology under DBT funded project.
10. Conducting Training Programs to Departmental Staff at Bivoltine Training School, Hindupur
11. I am one of the silkworm breeders for the APS45 x APS12 bivoltine hybrid which has been qualified in the Race Authorisation Test Phase VII.

Associated as Co-Principal investigator In Research Projects

1. Development of productive silkworm breeds suitable to eco-climatic conditions of Andhra Pradesh
2. Silkworm breeding for productivity improvement of silkSilkworm breeding for productivity and quality improvement of bivoltine silk.

(E) PARTICIPATION IN THE TRAINING UNDER EXPERTS:

Sl.No.	Training programs	Duration	Name of the organization
1.	Meticulous training under gone in Silkworm Genetics and Breeding, under the leadership of Dr. Chandrashekharaiyah , the former Director along with the senior Scientists of the institute.	1997- Till date	The Former Director APSSRDI Hindupur
2.	Undergone dedicated training in silkworm breeding by Prof. Wu Yucheng , the renowned and professional silkworm breeder from China	Six Months	Under the Seri-2000 project of the APSSRDI, Hindupur sponsored by Swiss Agency for Development and Cooperation (SDC)

(F). TECHNICAL TRAININGS UNDERGONE:

CERTIFICATE	AUTHORITY
Silk Reeling and Hand spinning	By Central Silk Technological Research Institute, At Demonstration Cum Training Centre, Hindupur
MS Office Automation(MS-word, MS-Excel, MS-PowerPoint)	The council of Education and Training First computers, Hindupur
English Type Higher	First class, Technical board, Andhra Pradesh

PUBLICATIONS

A. RESEARCH PAPERS:

1. Silkworm genetic stocks – an evaluatory analysis.
M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi** and J. Prasad
Bull. Indian Acad. Seric. 5(1): 9 -17 (2001)

2. Expression of hybrid vigour of silk productivity in silkworm *Bombyx mori* L. M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi** and J. Prasad
J. Exptl. Zoology 4(2): 317 – 321. (2001)
3. Multiple trait evaluation of bivoltine hybrids of silkworm (*Bombyx mori* L.). M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi** and J. Prasad
Int. J. Indust. Entomol. 5(1): 37– 43 (2002)
4. Combining ability of diallel crosses of bivoltine silkworm, *Bombyx mori* L. M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi**, J. Prasad and A.K. Goel. **Int. J. Indust. Entomol.** 10(1): 61-64 (2005).
5. Development of new productive silkworm (*Bombyx mori* L.) hybrid, APS₈₃ x APS₁₀₂. M. Ramesh Babu, **H. Lakshmi**, J. Prasad, J. Seetharamulu and Chandrashekharaiyah.
Int. J. Indust. Entomol. 11: 27-36 (2005).
6. Evaluation and selection of potential bivoltine parents of silkworm (*Bombyx mori* L) breeding. M. Ramesh Babu, **H. Lakshmi**, J. Prasad, J. Seetharamulu, Chandrashekharaiyah and A.K. Goel
Ind. J. Seric. 44(1): 82 – 92 (2005).
7. Identification of breeding resource material for the development of thermo-tolerant breeds of silkworm, *Bombyx mori* L. **H. Lakshmi** and Chandrashekharaiyah
J. Exp. Zool. 10(1):55-63 (2007).
8. Evaluation and selection of breeding resource material of bivoltine silkworm *Bombyx mori* L. suitable to tropical conditions. **H. Lakshmi** and Chandrashekharaiyah
Indian J. Agrl. Res. 42(4):235-243 (2008).
9. Identification of new cross breed APM1 x APS98 through manifestation of hybrid vigour. **H. Lakshmi**, M. Ramesh Babu, J. Prasad and Chandrashekharaiyah
Bull. Ind. Seric. Res. 12(2):1-11 (2008).
10. Effect of tukra (Mealy bug) infected mulberry leaves on the quantitative traits of new polyvoltine strain of silkworm, *Bombyx mori* L. C. Ramesha, S.V. Seshagiri, **H. Lakshmi**, S.S. Kumari and C.G.P. Rao
Journal of Entomology 6(4):198-205. (2009).
ISSN 1812-5670 (Academic Journals Inc. www.sciencealert.com)
11. Nutrigenetic traits analysis for identification of nutritionally efficient polyvoltine silkworm germplasm breeds. C. Ramesha, C.M. Anuradha, **H. Lakshmi**, S. Sugnana Kumari and C. Suresh Kumar.
Biotechnology 9(2): 131 – 140, 2010
12. Ramesh Babu, M.; **H. Lakshmi**; Prasad, J.; Seetharamulu, J. and Chandrashekharaiyah (2010) Studies on cocoon shape variability in different crosses of silkworm, *Bombyx mori* L.
J. Exp. Zool. 13(2):55-63.

B. RESEARCH PAPERS IN CONFERENCES/WORKSHOPS/SEMINAR:

13. Evaluation and Utilization of bivoltine silkworm germplasm.
Chandrashekharaiyah, M. Ramesh Babu, **H. Lakshmi** and J. Prasad
National workshop on management of sericultural germplasm for posterity.
held in July, at CSGRC, Hosur. (2000).
14. Cocoon filament size deviation in bivoltine breeds/hybrids of silkworm
Bombyx mori L.
M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi**, J. Prasad and J.
Seetharamulu
National conference on tropical sericulture for global competitiveness. 5-7th
November, CSR & TI, Mysore. Abs. No.SWI/R-3, pp. 33 (2003).
15. Combining ability analysis of diallel crosses in bivoltine silkworm *Bombyx mori* L.
M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi**, A.K. Goel and J.
Prasad
National conference on tropical sericulture for global competitiveness. 5-7th
November, CSR & TI, Mysore. Abs. No.SWI/R-3, pp. 26 (2003)
16. Evaluation and identification of bivoltine hybrids of silkworm, *Bombyx mori*
L. for tropical Conditions
M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi** and J. Prasad
**National Symposium (sponsored by the UGC) on 'Recent trends in
applied biology'** held at Avinashilingam Deemed University, Coimbatore,
28th-29th January, (2004).
17. Development of new productive bivoltine silkworm (*Bombyx mori* L.)
hybrid, APS₇₇ x APS₁₀₀.
Chandrashekharaiyah, M. Ramesh Babu, **H. Lakshmi**, J. Prasad and J.
Seetharamulu
Proceedings of V International China Silk Conference held on 21st – 24th
September at Soochow University, China. 255 – 272. (2004).
18. Evaluation and identification of promising bivoltine hybrids of silkworm
Bombyx mori L.
H. Lakshmi, M. Ramesh Babu, J. Prasad, J. Seetharamulu, and
Chandrashekharaiyah
**National Symposium (sponsored by the UGC) on 'Scenario of Sericulture in
India'** held at Sri Padmavathi Mahila Viswa Vidyalayam, Tirupathi, 25th – 26th March
(2005).
19. Breeding of new bivoltine silkworm (*Bombyx mori* L.) hybrid, APS₄₅ x APS₁₂ for
productivity and quality.
Chandrashekharaiyah, M. Ramesh Babu, J. Prasad, **H. Lakshmi** and J.
Seetharamulu.
**Asia Pacific Congress of Sericulture and Insect Biotechnology (Republic of
Korea)** (2006).
20. Conservation and utilization of sex limited bivoltine silkworm genetic resources.

Ajay Kumar Goel, Y. Umamaheshwar Rao, **H. Lakshmi**, K. Ibrahim Basha, V. Sivaprasad and Chandrashekharaiyah.

National Workshop on SERI- BIODEVERSITY CONSERVATION. Held at CSGRC, Hosur Tamil Nadu on 7th – 8th March, pp:112-117 (2009).

21. Studies on Genetic Divergence in Bivoltine Genotypes of silkworm *Bombyx mori* L.
H. Lakshmi, J. Seetharamulu, M. Ramesh Babu, Chandrashekharaiyah, V. Siva Prasad, Ajay Kumar Goel and P.J. Raju
Paper presented in National Seminar on 'Zoology, Life Processes and Nanotechnology' 8th to 10th February, 2010, Department of Zoology, Goa University, Goa.
22. Development of high temperature tolerant bivoltine breeds of silkworm *Bombyx mori* L.
H. Lakshmi, J. Seetharamulu and P.J. Raju
Paper presented in workshop on state sericulture, technical compendium held by Ministry of Textiles, Orissa, Bhubaneswar on March, 24-26th 2010.

C. POPULAR ARTICLES IN GENERAL SERICULTURE (ENGLISH)

1. Mulberry - An ideal resource for potential exploitation of wide range biotechnological products.
Ramesha, C., Ibraheem Basha, K., **Lakshmi, H.**, Seshagiri, S.V., Kiran Kumar, P. K.
Indian Silk 41(11): 5 – 7, 2003.
2. Silk filament – its pharmaceutical application.
C. Ramesha, C. Suresh Kumar, S.V. Seshagiri, K. Ibrahim Basha,
H. Lakshmi, C.G.P. Rao and Chandrashekharaiyah.
Indian Silk 44(2): 15 & 19. 2005.
3. Transgenic silkworm - its biotechnological applications.
C. Ramesha, C. Suresh Kumar, **H. Lakshmi**, S.V. Seshagiri, K. Ibrahim Basha,
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D. POPULAR ARTICLES IN GENERAL SERICULTURE (TELUGU)

4. Pattupurugula pempakamlo 'uzi' niyanthrana paddathulu (Telugu)
M. Ramesh Babu, Chandrashekharaiyah, V. Siva Prasad, **H. Lakshmi**, A.K. Goel and J. Prasad.
Annadata, January, 2002. pp: 11-12.
5. Bivoltine pattulo chawki purugula pempakam – Melakuvalu (Telugu)
M. Ramesh Babu, Chandrashekharaiyah, V. Siva Prasad, **H. Lakshmi** and J. Prasad
Annadata, February, 2002. pp:9-10.
6. Varshakalamlo Bivoltine pattupurugula pempakam – Jagrathalu (Telugu)
M. Ramesh Babu, Chandrashekharaiyah, **H. Lakshmi**, V. Sivaprasad, J. Prasad and C.G.P. Rao.
Annadata, June 2002. pp: 64 – 66.
7. Nanyamyna bivoltine vithana pattugulla uthpathilo melakuvalu (Telugu)

- M.Ramesh Babu, **H.Lakshmi**, J. Prasad, J. Seetharamulu,
Chandrashekharaiyah
Annadata, August, 2003, pp: 37-39.
8. Noothana Bivoltine Sankarajathi pattu rakalu Hemavathy marihyu
Kalpatharuvu
M.Ramesh Babu, Chandrashekharaiyah, J.Seetharamulu, **H.Lakshmi** and
J. Prasad
Annadata, January 2003, pp: 23 – 24.
9. 'Nanyatha gala bivoltine vithana goolla uthpathiki patinchavalasina sankethika mariyu
yajamanya paddathulu'(Telugu)
M. Ramesh Babu, **H. Lakshmi**, J. Prasad and Chandrashekharaiyah
Annadata, July, 2003.
10. 'Mulberry saguku anuvyna nela rakalu – vati pramukhyatha' (Telugu)
M. Ramesh Babu, **H. Lakshmi**, J. Prasad, J. Seetharamulu and
Chandrashekharaiyah.
Annadata July, 2004.
11. 'Mulberry saguku jeevana eruvula viniyogamu'(Telugu)
M. Ramesh Babu and **H. Lakshmi**
Annadata, March, 2008.

E. TEXT BOOKS

1. Ramesha, C.; Suresh Kumar, C; **Lakshmi, H.**; Pushpalatha, C.; Sugnana Kumari,
S. 2009. Chapter no. 5 on 'Biotechnological Prospectives of Mulberry and Silkworm'
contributed for text book entitled "Application of Biotechnology in Sericulture"
Edited by Dr. Venkatesh Kumar, R. Published by **Stadium Publication Co. Pvt. Ltd.** New
Delhi. pp. 43-70.

Place: Berhampore
Date:

Signature of the Investigator

Annexure-V

2. EQUIPMENT & ACCESSORIES TO BE UTILISED FOR THE PROJECT:

Sl. No	Name of equipment / accessories	Make	Quantity (model)	Funding Agency	Remarks
1.	Refrigerated Centrifuge (high speed)	Sorvall	1	CSB	
2.	Micro Centrifuge	Hermile	1	CSB	
3.	Vertical Gel Electrophoresis system	Omega, Japan	1	CSIR	
4.	Gel Documentation system	UVP	1	CSB	Not repairable
5.	Gel Dryer	Pharmacia-LKB	1	CSB	
6.	Thermo-controlled Water Circulating Bath (-20° to 100°C)	Pharmacia-LKB	1	CSIR	
7.	Ice Flakers	Simag	1	CSIR	
8.	Cocoon weighing balance	Anamed	1 + 2	CSIR+ CSB	
9.	pH Meter	Thermo	1	CSB	
10.	Electronic top loading balance	Sartorius	1	CSB	
11.	Chemical balance	Sartorius	1	CSB	
12.	High precision Incubator	Remi	1	CSB	
13.	Homogeniser	Remi	1	CSB	
14.	Water distillation plant	Local made	1	CSB	
15.	Micro pipettes	Eppendorf, Tarson	6	CSIR	
16.	Dissecting zoom stereo microscope	Leica	1	CSB	
17.	PCR	Eppendorf	1	CSB	
18.	DNA Hybridisation Chamber with Shaker	Binder	1	CSIR	
19.	UV Spectrophotometer	Shimadzu	1	CSB	
21.	Liquid Column Chromatography	Pharmacia	1	CSB	
22.	Air Conditioner	Voltas	7	CSB	
23.	Refrigerators	LG	3	CSB	
24.	Computer	HCL	1	CSB	
25.	- 86°C Deep Freeze		1	CSB	
26.	-35°C Deep Freeze	Remi		CSB	

PART VI: DECLARATION / CERTIFICATION

It is certified that

- a. The research work proposed in the project does not in any way duplicate the work already done or being carried out elsewhere on the subject.
- b. The same project has not been submitted to any other agencies for financial support.
- c. The emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute / university or as per the Ministry of science & technology guidelines (Annexure – III).
- d. Necessary provision for the project will be made in the Institute in anticipation of the sanction of the scheme.
- e. If the project involves the utilization of genetically engineered organism, it is agreed that we will ensure that an application will be submitted through our institutional bio-safety committee and we will declare that while conducting experiments, the bio-safety committee we will declare that while conducting experiments, the bio-safety guidelines of the Department of Biotechnology would be followed in toto.
- f. If the project involves field trials / experiments / exchange of specimens etc. we will ensure that ethical clearances would be taken from the concerned ethical committees / competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.
- g. It is agreed by us that any research outcome or intellectual property right(s) on the invention (s) arising out of the Project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance. Department of Expenditure, as contained in annexure–V.
- h. We agree to accept the terms and conditions as enclosed in Annexure – IV. The same is signed and enclosed.
- i. The Institute agrees that the equipment, the basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigators throughout the duration of the project.
- j. The Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Executive Authority of
Institute with Seal
Date:

Signature of Project Coordinator

[Applicable for Inter-Institutional Projects Only]
Date

1. Signature of Principal Investigator

Date:

2. Signature of Co-Investigator-1

Date:

3. Signature of Principal Investigator -2

Date:

4. Signature of Co-Investigator-3

Date:

5. Signature of Co-Investigator-4

Date: