AIB 3619

Development of silkworm (*Bombyx mori* L.) Congenic Breeds from a Gene Pool with higher Genetic Plasticity (Phase-II)

DURATION: JULY, 2017 TO JUNE, 2020

Central Silk Board
Ministry of Textiles (Government of India)
BTM Layout, Madivala
Bangalore – 560 068

Principal Investigator: *Dr. A. K. Verma*
(*Scientist-D*)

Silkworm Breeding & Genetics Laboratory
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 Sericultural 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 Sericultural Research &
Training Institute,
Berhampore - 742101, Murshadabad, West Bengal.

4. Project Title:

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

5. Category of the Project: **AIG - 02**

6. Specific Area: **Silkworm Improvement**

7. Duration: **3 Years**

8. Total Cost: **11.416 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 D6*p* 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 JPN*p* from M6DP*C* having high survival), KPGA, RSJ-14, DUN-21, CSR-2 and APS- 45 (Annexure-III) were 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 was 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 were screened having genetic plasticity in the gene pool for a target trait under variable climatic situation. These screened multivoltine and bivoltine breed will be used further to develop **Congenic 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-III). Each and every step of breeding will be done to understand the diversity or similarity of the target characters and its' persistence in developed breeds which may be considered as one of the factor 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.

i) **Name:** **Dr. N. Chandrakanth**
ii) Date of birth: 24.04.1986
III) Sex**:** Male
iv) Indicate whether Principal Investigator / Co-investigator**:** **Co- Investigator-1**
v) Designation & Department: Scientist - B
vi) Institute / University Address: Silkworm Breeding & Genetics Section
Central Sericulture Research & Training Institute,
Berhampore- 742101,Murshidabad, West Bengal.

i) **Name:** **Shri N.B.Kar**
ii) Date of birth: 1959
iii) Sex **:** Male
iv)Indicate whether Principal Investigator/ Co-investigator**:** **Co- Investigator-2**
v) Designation & Department**:** Scientist - D
vi) Institute / University Address: Reeling & Spinning Section
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- Three project
Co- Investigator: 2 - Four

14. Proposed **Research Fellows**: **One**

Justification for Research Fellow:

The New Project entitled "Development of silkworm *Bombyx mori* L. Congenic breeds from a gene pool with higher genetic plasticity Phase-II of AIB-3480" is approved by 45th RAC in January, 2017 and the same is being sent to CO., Central Silk Board, Bengaluru for coding.

The project is aimed to develop Bivoltine Congenic breeds with high survival and Multivoltine Congenic breeds with high shell weight. At present our main focus is **Climate resilient sericulture-i.e. Development of suitable Breeds to meet environmental challenges**. Again these breeds can be released in the field without hybridization having better and sustainable cocoon yield compared to the hybrids.

In this project, it is planned to develop up to RBL4-Recurrent Backcross Line 4 (Congenic Line). One cycle i.e., RBL1 requires crossing (one time) followed by Back crossing (five times) and Sib-mating (five times)- a total of 11 generation per cycle or about a total of 36 to 44 generations. Selection right from egg laying for blueing time &hatching, for larval marking & scotophase period, single cocoon analysis on 5th-6th day of cocooning of each cocoons of all the lines along with identification of biochemical / molecular markers associated with the target traits which are very tedious, specialized and time-bound task, will be undertaken.

Furthermore, the PI and other associated scientists of this project are already involved in more than 3 projects each and cannot afford much time to carry out such a huge work. In view of the above, it is requested to consider the proposal of a JRF for the project period (Three years) for successful implementation of the project. The tentative budget for JRF will be 5.016 lakhs.

PART 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 36ºC (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). Even B.Con.1 & B.Con.4 individually without hybridization fetched more than 75kg/100dfls in south India which is better than double hybrid (NSSO-2016).

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:

Eastern and North Eastern region with special 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 due to high rainfall and fertility of soil. But, the practice of bivoltine rearing or productive silkworm breeds or their hybrids is very restricted due to 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 (> 35^0 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 (V_3) 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 develop 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 Silkworm Hybrid Authorization Programme (MSRAP). B.Con.1 x B.Con.4, a bivoltine congenic hybrid is also showing excellent result in the field.

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 can be used for identifying diversified region in biochemical and DNA level as congenic breed provides a root of quickly identifying marker linked to traits.

In the Phase-I of this project, multivoltine and bivoltine convergent gene pool have been developed with genetic plasticity for high shell weight and horizontal tolerance respectively (Concluding report already submitted). Now in the proposed project (Phase-II), these convergent lines will be utilized 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 considered 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 (>35^0 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 $M_{12}W$ 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 P_1, 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 of 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 chance 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.

15.4 OBJECTIVE:

- Development 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/ developed Converged Gene pool as parent for high cocoon shell weight and bivoltine breed for horizontal tolerance.

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 Inbreed 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 (V_2) 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 -III**). Following this breeding approach survival character from multivoltine (V_3) CB5 breed was introgressed to JPN bivoltine (V_2) 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, D6*p* 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 (V_2) breeds are giving better survival in comparison to traditional any bivoltine (V_2) 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 (V_2) JPN breed was introgressed to (V_3) CB5 by developing high cocoon shell weight V_3 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 D6*p* 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 (V_3) 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 (V_3) breeds having cocoon shell weight 0.115g to 0.135g (> Nistari). Developed (V_2) breeds B.Con.1, B. Con.4 and (V_3) 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 V_3 x V_3 or V_3 x V_2 and V_3 Congenic breed x V_2 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±1°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 P_1 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 farmer's level throughout the year will be possible to boost the mulberry raw silk production under 11^{th} year plan.

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

Developed Congenic breeds to be exploited as a P1 breeds at farms and farmers level to over come the scarcity of multi x bi or bi x bi dfls during commercial seasons. These breeds can also be utilized without hybridization in the field without any effect on the production.

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 other part.

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

Review not required as it is Phase-II of the project-AIB-3480. 45th RAC held on 17.01.17., **recommended** for continuation of the project as Phase-II for another three years, which will be sent to the Central Office for coding.

17. WORK PLAN

17.1. Methodology:

Six Bivoltine and Six Multivoltine Breeds/ Strains from different geographical zones of India **(Annexure – I)** are collected and conventional silkworm rearing method had been followed for assessment of quantitative and qualitative characters in West Bengal climatic condition. Thereafter convergent breeding approach **(Annexure-II)** was adopted to make **broad genetic base to bring genetic plasticity** in bivoltine for high cocoon shell weight and in multivoltine for high survival- pupation percentage.

Thus, in the **Project AIB-3480**, the following convergent lines have been developed:-

Bivoltine Convergent Line

Six ways cross *i.e.,* (B.Con.4 x CSR-2) x (Dun-21 x KPG-A) x (RSJ-14 x APS-45)-raised as **converged gene pool** for high Shell weight after strict selection and assimilation of target character for **12generations** and two lines were isolated as:

1. **Plain (*p*) Larvae, Faint Constricted, White (*c*) colour Cocoon**
2. **Marked (+p) Larvae, Faint Constricted, White (c) colour Cocoon**

Rearing performances of the above breeds are given below:

Table-1: Rearing Performance of Bivoltine convergent lines (B.Con.4 x CSR-2) x (DUN21 x KPG-A) x (APS-45 x RSJ-14).

Character	Fec	Hat %	Yield/ 10000 lar. (no.)	Yield/ 10000 lar. (wt.kg.)	SCW (g)	SSW (g)	Shell %
Plain (*p*) Larvae, Faint Constricted, White (*c*) colour Cocoon	532	93	9100	14.13	1.565	0.288	18.40
Marked (+p) Larvae, Faint Constricted, White (c) colour Cocoon	542	93	9033	14.57	1.569	0.285	18.16

Multivoltine Convergent Line

Six Way cross *i.e.,* (Nistari +*p*) x Cambodge) x (M.Con.4 x PM) x (MH1 x Sarupat) – raised as **converged gene pool** for horizontal tolerance (survival) after strict selection and assimilation of target character for **15 generations** and three lines were isolated as:

1. Plain (*p*) Larvae, Yellow (*C*), Oval shape Cocoon

2. Plain (*p*) Larvae, White (*c*), Oval shape Cocoon

3. Plain (*p*) Larvae, Light Greenish(*Gr*) Oval shape Cocoon

Rearing performances of the above breeds are given below:

Table-2: Rearing Performance of Multivoltine convergent lines (Nistari+*p* x Cambd) x (M.Con.4 x PM) x (Sarupat x MH1)

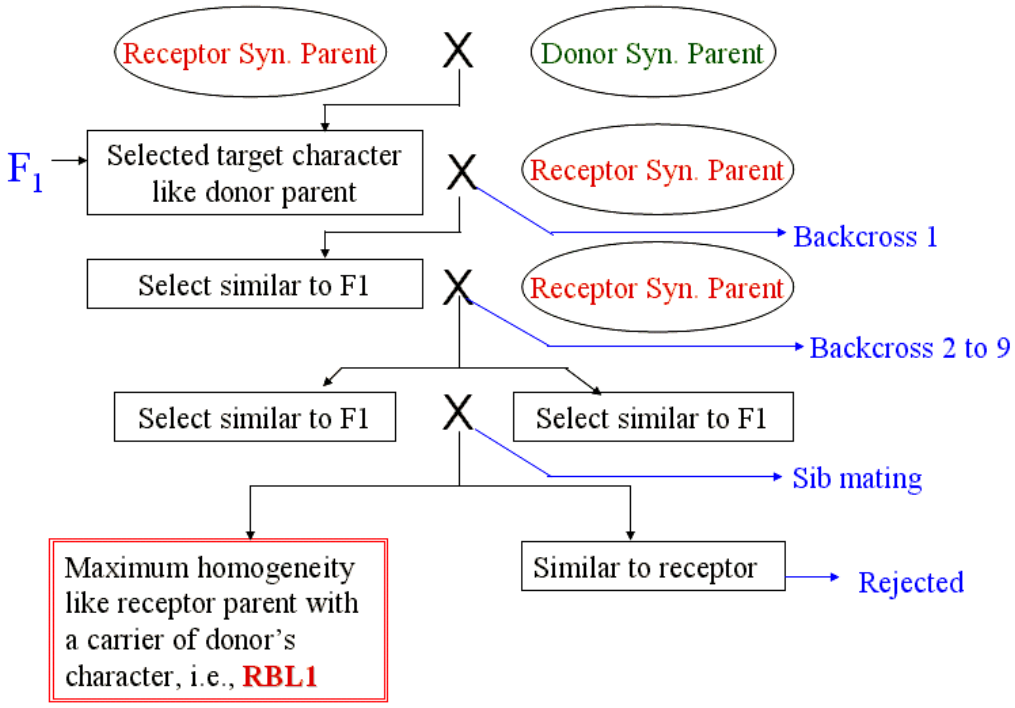
Character	Fec	Hat %	Yield/ 10000 lar. (no.)	Yield/ 10000 lar. (wt.kg.)	SCW (g)	SSW (g)	Shell %
Plain (*p*) Larvae, **Yellow (*C*), Oval Cocoon**	517	97	9433	12.03	1.306	0.208	15.92
Plain (*p*) Larvae, **White (*c*),Oval Cocoon,**	502	97	9467	11.70	1.399	0.211	15.08
Plain (*p*)Larvae, Light **Greenish (*Gr*) OvalCocoon**	525	95	9533	11.67	1.348	0.201	14.90

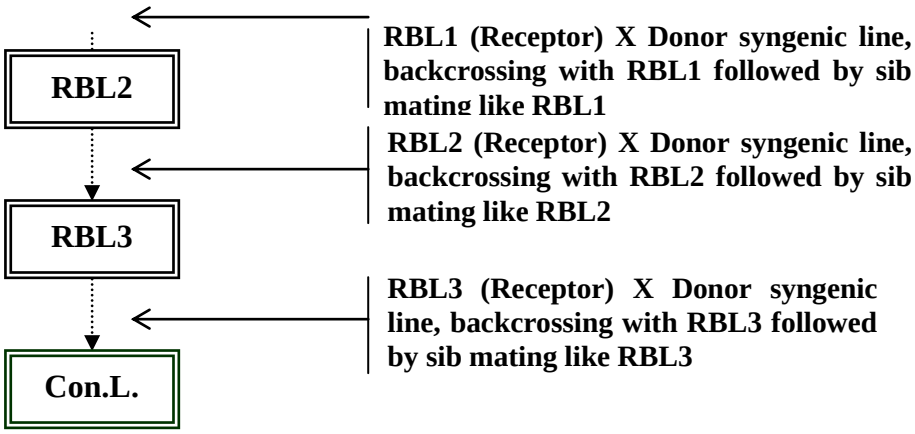
The above bivoltine and multivoltine convergent lines will be utilized **as parents** to develop multivoltine **congenic lines with high shell weight** (>0.24g) and bivoltine congenic lines with high survival (>90%) in this **Phase-II of the project** by following the methodology depicted in **Annexure-III** for development of congenic line**, as per the RAC decision.**

For the development of Bivoltine congenic line, the bivoltine convergent line will be used as receptor parent and multivoltine convergent line as donor parent and for Multivoltine congenic line vice-versa.

Annexure-III

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





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

17.2. Organization of Work Elements

Name	Work to be done
Dr. Kanika Trivedy	Over all coordination and guidance
Dr. A. K. Verma Principal Investigator	Development of productive and tolerant Congenic Breeds, compilation and interpretation of data, submission of report time to time and final report preparation.
Sri N. Chandrakanth Co-Investigator-1	Associated with above work, data compilation and to assist in report and final report preparation
Mr.N.B.Kar Co-Investigator-2	Associated with above work for post cocoon Analysis

THE EXPERIMENTS ASSOCIATED WITH THIS PROJECT ARE AS FOLLOWS:

EXPERIMENT TITLE	**: Development of productive and tolerant Congenic Breeds.**
OBJECTIVES	**:** To develop productive and tolerant Congenic Silkworm Breeds.
STARTING DATE	: July-2017 (Phase-II)
COMPLETING DATE	: June-2020
METHODOLOGY	Congenic breeding approach (Chattopadhyay et al 2001a, b., 2005 **(See Annexure-III)**

ACTIVITY	**TIME EXPECTED**	
	DATE OF STARTING	**DATE OF CLOSING**
Development of Congenic Bivoltine and Multivoltine breeds through development of RBL1→RBL2→RBL3→Con. Line.	From July, 2017 (Phase-II)- details given below.	June-2020

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	
1.	Raising of RBL1	July-2017	April-2018	Multivoltine RBL1 with improved shell weight and bivoltine with improved survival
2.	Raising of RBL2	May--2018	Feb.-2019	Multivoltine RBL2 with better shell weight and bivoltine with better survival than RBL1
3.	Raising of RBL3	March-2019	Dec.-2019	Multivoltine RBL3 with better shell weight and bivoltine with better survival than RBL2
4.	Development of productive and tolerant Congenic breeds	From Jan., 2019 or after the development of RBL3	June-2020	Multivoltine Congenic breed with high shell weight nearer to bivoltine donor and Bivoltine congenic breed with high pupation rate nearer to multivoltine donor parent.

PART IV: BUDGET PARTICULARS:

18. **BUDGET (in Lakhs):**

C. BUDGET ESTIMATES: SUMMARY

ITEM					
BUDGET					
	1st Year	2nd Year	3rd Year		Total
A. Recurring					
1. Remuneration /Salaries	1.584	1.584	1.848		5.016
2. Consumables(including chemicals)	1.00	1.00	1.00		3.00
3. Travel	0.20	0.20	0.20		0.60
4. Other Costs	0.10	0.10	0.10		0.30
B. Non-recurring A.C.-1,Microscope-1, Refrigerator-1 & Cocoon weighing Machine-1	2.5	-	-		2.50
Grand Total (A + B)	4.884	2.384	2.648		**11.416**

BUDGET REQUIREMENT FOR MANPOWER OF PHASE-II of AIB-3480

Designation Number of Persons	**1st year**	**2nd year**	**3rd year**	**Total**
JRF (1person)-Proposed Consolidated @Rs.12000/month	1.44	1.44	1.68	4.56
10% HRA for two years & third year	0.144	0.144	0.168	0.456
Grand Total	**1.584**	**1.584**	**1.848**	**5.016**

DETAIL BUDGET FOR TRAVEL:

ITEM BUDGET	1st Year	2nd Year	3rd Year		Total
Travel 1. Local	0.05	0.05	0.05		0.15
2. Out Station	0.15	0.15	0.15		0.45
TOTAL	0.20	0.20	0.20		0.60

DETAIL BUDGET FOR OTHER COSTS:

ITEM BUDGET	1st Year	2nd Year	3rd Year		Total
a) Contingencies	1.00	1.00	1.00		3.00
b) Others	0.10	0.10	0.10		0.30
Total	1.10	1.10	1.10		3.30

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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 (V_2)

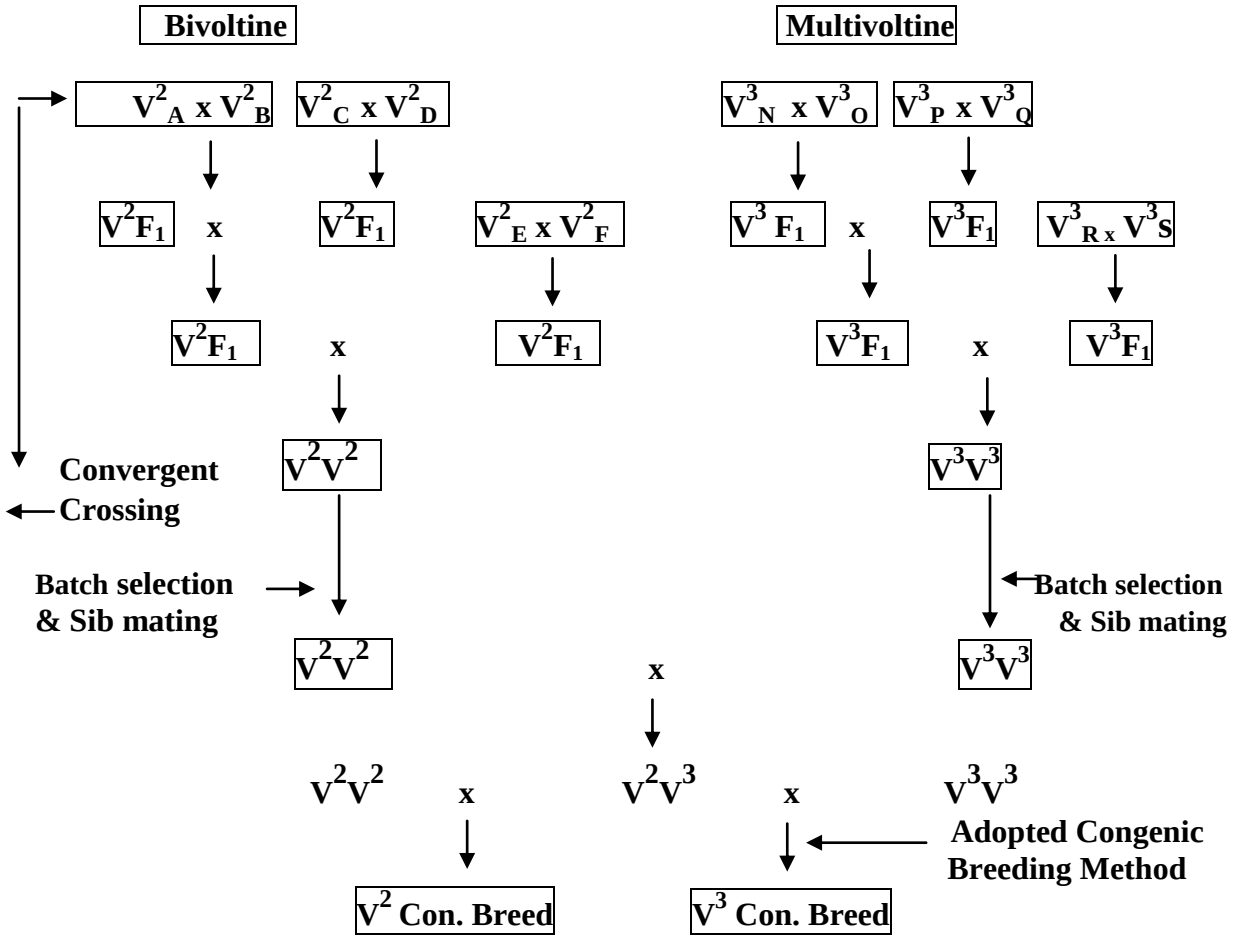
Sl. No	Breed's Name	Origin	Cocoon shape	Cocoon Colour	Shell Wt.	Shell %	Pupae%
1	**B.Con-4**	CSR&TI, Berhampore (Variable climate)	Oval slightly Cons.	Off white	0.318	18.06	Un.F- 50.8 Fav-73.0
2.	**KPG-A**	RSRS Kalimpong (Congenial climate)	Oval	White	0.275	21.90	85.70
3.	**RSJ-14**	RSRS Jammu (Congenial 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 (V_3)

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 Pradash	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

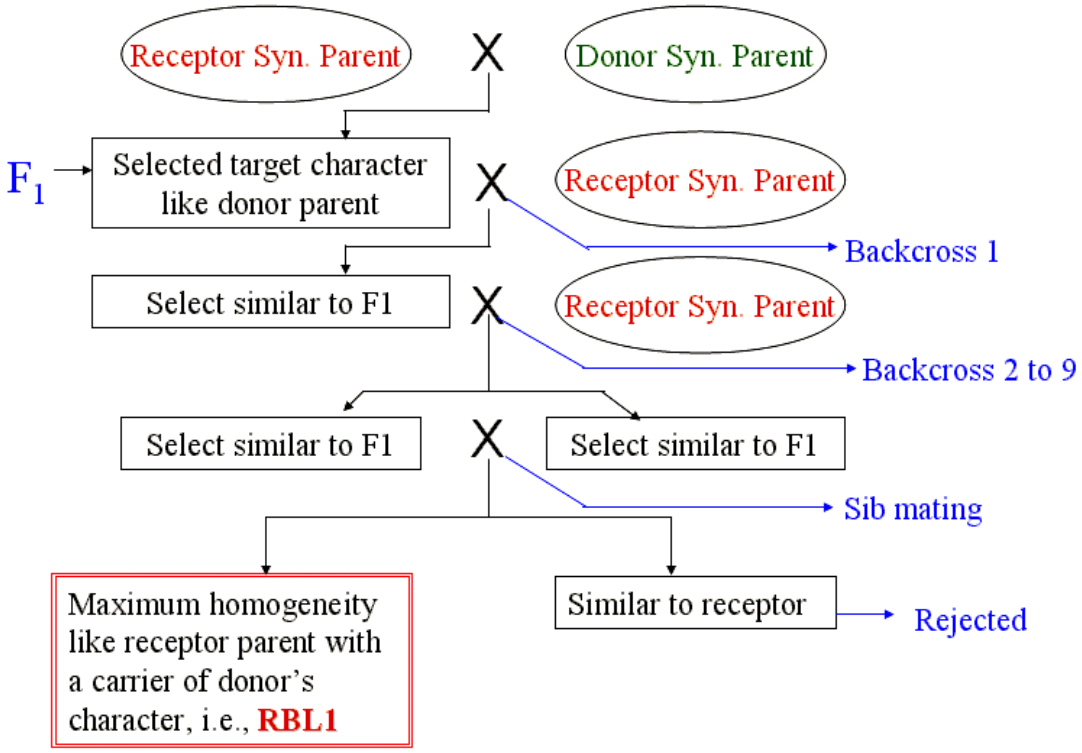
Annexure-II

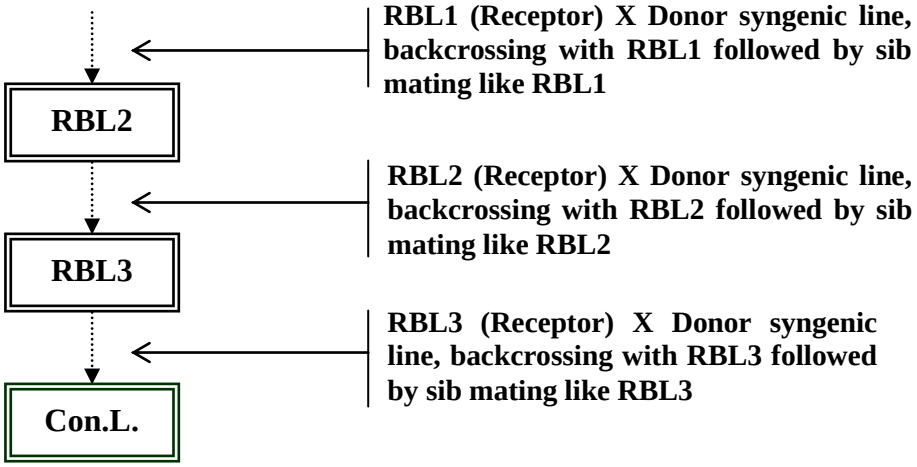
Scheme for Development of a Line / Breed having Genetic Plasticity



Annexure-III

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

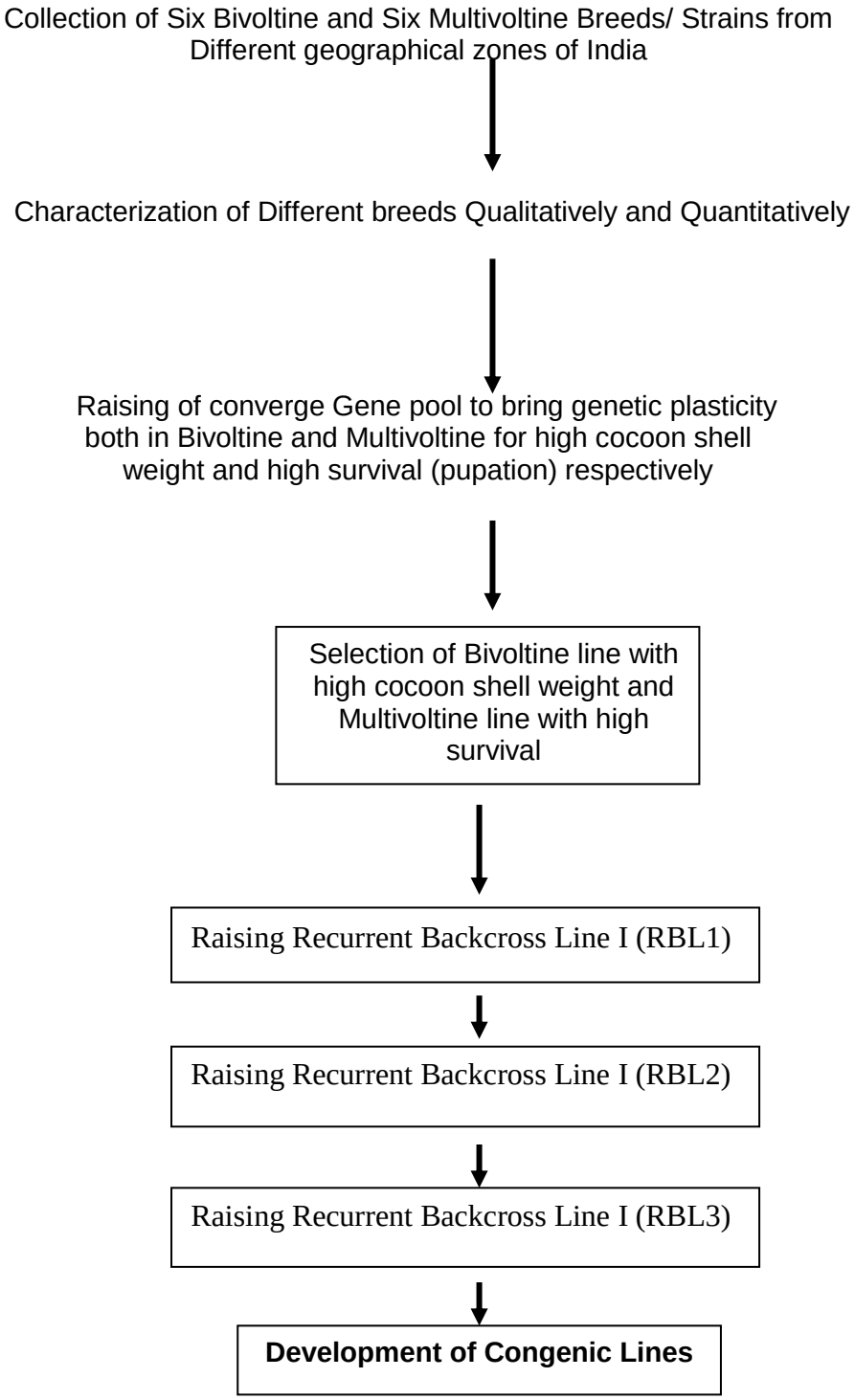




*** Number of crossing with donor depends upon the transgression of target trait**

ANNEXURE-IV

FLOW CHART



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.	- 86^0C Deep Freeze		1	CSB	
26.	-35^0C Deep Freeze	Remi		CSB	

PART VII: BIODATA OF PRINCIPAL INVESTIGATOR

1.	Full Name (in Block letters)	DR. ANIL KUMAR VERMA
2.	Designation	Scientist-D.
3.	Department/Institute/University	Silkworm Breeding Section, Central Sericultural Research & Training Institute, Berhampore(WB)-742101
4.	Date of birth	28.12.1960.
5.	Sex	Male.

6. Education (Post Graduation onwards & Professional careers)

Name of the University	Degree passed	Year of passing	Subjects taken with specialization	Class / Division
1. University of Kalyani, Nadia, West Bengal.	M.Sc.	1983	Zoology, Spl.: Entomology.	I
2. Bidhan Chandra Krishi Viswavidyalaya, West Bengal	Ph. D	1990	Title of the Thesis- Studies on **whitefly** as vector of plant viruses in West Bengal. Under Prof. Sankar Mukhopadhyay and Prof. S.S.Ghatak	-

7. Position held/research experience in various Institutions:

Employer	Designation of the post held	Date of joining	Date of leaving
Central Silk Board	Senior Research Assistant	12.10.1990	
-do-	Senior Research Officer	12.10.2000	
-do-	Scientist-C	12.10.2006	
-do-	Scientist-D	01.01.2015	

8.	Memberships/Fellowships:	NASSI,
9.	Patents/ Breeds & Hybrids authorized:	5 Hybrids developed and authorized
10.	Publications (numbers only)	24 Papers and 38Technical Reports

11. TRAINING UNDERGONE:

COURSE NAME/ SUBJECT	**ADDRESS OF INSTITUTE**	**DURATION**	**PERIOD**
1.Refressor training course programme (Non-mulberry)	CTRTI, Ranchi.	26 days	22.6.94 to15.7.94
2.S.W.Seed production techniques (Mulberry)	SSTL, Kodathi	15 days	15.11.94 to 29.11.94
3.Statistical methods for Sericultural research.	CSB, Bangalore	4 days	19.5.03 to 22.5.03
4.Computer Application	ITI, Murshidabad	6 days	07.7.03 to 12.7.03
5.Right to Information Act-2005	R.O. New Delhi	2 days	24.02.10-25.02.10
6.Disciplinary Proceedings Training	CSRTI, Berhampore	4 days	16.11.10-19.11.10

12. Project(s) submitted / being pursued / carried out:

Sl. No.	Title of the project	Funding agency	Duration From To
1	Utilization of Syngenic lines for improvement of shell weight and survival in silkworm, *B. mori*. L	CSB	Dec., 1998 to Dec.,2004
2	Introgression of higher shell weight, higher survival character/gene through the development of multivoltine and bivoltine congenic breeds and identification of biochemical marker in silkworm, *B. mori*. L.	CSIR, Delhi	April, 2002 to March,2005
3	On farm trial of congenic silkworm hybrids for commercial exploitation	CSB	Sept.,2004-Dec06
4	On Farm trial of evolved bivoltine and multivoltine Congenic breeds and their hybrid performance at farmer's level (In collaboration with DOS, W.B.	CSB	Dec' 05-Nov' 2007
5	Mulberry Silkworm race Authorization Programme (MSRAP) – Phase- vii	CSB	2005-2008
6	Multi location Trial of New Silkworm Breads/Hybrids at Farm Level	CSB	Dec. 2007-Mar. 2010
7	Institute Village Linkage Programme (IVLP)	CSB	Ph.I:07-10
8	Study on the efficacy of newly developed Bed disinfectant (Sericillin) in hot spot areas for the control of Muscardine disease of silkworm, *B. mori* L..	CSB	PhII:10-13 Aug.2010--Jul2012
9	Validation trial of the Ready Reckoner of sulphur fertilizer application for obtaing targeted yields of mulberry	CSB	2010-12
10	Validation trial of technology for Prevention of Gattine disease in hot spot areas in silkworm *B. mori* L.	CSB	2012-14
11	Development of Multivoltine silkworm breeds with high shell percentage and neatness of silk filament	CSB	Jul, 2013 - Jun2016
12	Post Authorization Trial of Silkworm hybrids in Eastern and North-Eastern India	CSB	Jun,2012 - Dec2014
13	Survey and surveillance of silkworm diseases in traditional districts of West Bengal	CSB	Jul,2013-Jun,2016
14	Silkworm disease monitoring of seed and commercial crop rearing of West Bengal	CSB	Apr,2013-Mar,16
15	Maintenance of Multivoltine and Bivoltine Germplasm.	CSB	Continuous.
16	Pre-Authorisation Trial of Silkworm hybrids in Eastern and North-Eastern India	CSB	Jun,2014-Dec2016
17	Development of silkworm *Bombyx mori* L. Breeds from a gene pool with higher genetic plasticity	CSB	Sept.2012 – Aug.2016
18	Development of multivoltine congenic / NIL breed of silkworm *Bombyx mori* L. Through introgression of Id gene and its use	CSB	Jun.2014 - May2017

13. Highlights of outcome / progress of the project(s) handled during the past 10 years, their outcome and utilisation (in 200 words):

Project	Outcome	Utilisation
1. Utilization of Syngenic lines for improvement of shell weight and survival in silkworm, *B. mori*. L	Isozyme based seven multivoltine and one sex linked bivoltine syngenic lines, four high cocoon shell weight multivoltine congenic breeds & four high survival bivoltine congenic breeds developed. Biochemical marker for high shell weight and high survival identified	The hybrids of the developed congenic breeds are under Post Authorization Trial.
2. Introgression of higher shell weight, higher survival character/gene through the development of multivoltine and bivoltine cogenic breeds and identification of biochemical marker in silkworm, *B. mori*. L.	Isozyme based three syngenic lines of Nistari and one high survival bivoltine congenic breeds developed. Two biochemical marker identified similar to above project.	The hybrids of the developed breeds are under Post Authorization Trial.
3. Institute Village Linkage Programme (IVLP)	Mulberry leaf yield (MT/ha/year) increases from initial 8 to 10.47(30.86%). Average Mulberry holding (in acre) increases from initial 0.5 to 0.66(32.00 %.). Average rearing capacity (DFLs/farmer/crop) increases from initial 75 to 125 (66.66 %.). Cocoon yield/ 100 DFLs (kg.) increases from initial 28.99 to 36.62(26.31 %.)	Plantation of High Yielding Variety like S1635. Plant to plant & row to row spacing- 2 ft.X 2 ft. Use of Plant Growth Hormone like Morizyme-B. Use of Vermicompost. Use of Biofertilizers like Nitrofert and Phosphofert to reduce the application of chemical nitrogen and phosphorus. Use of promising hybrids in place of existing one. Use of Bleaching Powder for general disinfection and Labex as bed disinfection. Use of dichlorovos for control of Whitefly infestation.
4. Validation trial of the Ready Reckoner of sulphur fertilizer application for obtaing targeted yields of mulberry	Soil from individual farmers analized to determine the extent of sulphur, based on this recommendation for sulphur application has been worked out.	Farmers are utilising this recommendation for use of sulphur in their field

Introduced 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., V^3 CB5-Con.Ow, V^3 M6DPC-Con.C, V^2 D6p-Con.Ow, V^2 D6p- Conc., V^2 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.**

It has been identified **224kDa Protein as a biochemical marker at pH-8.5 for high survival.** The apparent native protein in haemolymph is the possessor of α-Est s are exclusively present in multivoltine.

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

β -amylase presence in haemolymph and digestive of Silkworm, *Bombyx mori* L. and **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 ± 1°C), which has been considered as one of the molecular factor for thermo-tolerance.**

Specific **Isozyme possessor native proteins** are associated with **non-hibernation and hibernation character** of silkworm has been identified some (CSIR Final report)

In the Project **AIB-3501**, **seven multivoltine lines** with high SR% (**more than 17%**) and high neatness (**more than 80 points**) have been developed.
Besides, best two Multi x Multi and two Multi x Bi hybrids are also identified.

In the Project **AIB-3480**, **two six way bivoltine converged gene pool for high Shell weight and three multivoltine converged gene pool for high survival are developed**.
These lines will be used to develop 2 multivoltine congenic lines with high shell weight (>0.24g) and 2 bivoltine congenic lines with high survival (>90%) in Phase-II.

14. PUBLICATION:

1. Ph.D. Work guided-1
2. Book-3
3. Leaflet-9
4. Paper published-24

LIST OF IMPORTANT PUBLICATIONS

1. 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 thermo stable esterase in tropical silkworm *Bombyx mori* L., ***Insect Biochem. Mol. Biol. (USA),* 31: 1191-1199.**
2. 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.**
3. 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.**
4. **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): 21-27.**
5. Chattopadhyay, G. K, **Verma, A. K**, Sengupta. A. K, Das. S. K and Raje Urs, S. (2004) α and β-Amylase isozyme expresser native proteins in tropical silkworm *Bombyx mori* L. ***Inter. J. of Indust. Ent. (IJIE), Korea.* 8(2): 189-194**
6. **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**
7. 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.**
8. **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.**
9. 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.**
10. Chattopadhyay, G. K.; **Verma,A.K;** Das N.K. Saratchandra.B,: Bindroo, B.B and Saha ,A.K (2013) Performance of parents, their Syngenic lines, Congenic breeds and hybrids of silkworm, Bombyx mori ,L – A Comparison. **J. Exp. Zool, Vol.16, No.2, pp. 509-518.**
11. Chattopadhyay, G K, **Verma**, **A K,** Saha, A K and Nirmal Kumar, S (2015) Specific difference among isozyme possessornative proteins in haemolymph of tropical multivoltine, bivoltine and developed congenic breeds of silkworm (Bombyx mori L.) accepted for publication in *Biochem. Cell. Arch.* Vol. **15**, No.2, 549-555, 2015.
12. Suresh Kumar, N, **Verma, A.K**. and Saha, A.K. (2016) Breeding strategies for development of silkworm breeds/ hybrids foe Eastern and North-Eastern India. In " Proceeding of the Silkworm Breeders' Meet 2015 22th September 2015, CSRTI, Mysuru", pp. 14-20.
13. **Verma,A.K,** Chatterjee, G.K., Kar, N.B. Saha ,A.K., and Suresh Kumar, N. (2016). Esterase α, β - the biochemical markers for quantitative and qualitative traits of Silkworm, *Bombyx mori* L**. *Sericologia,*** 56(2): 94-102.

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PART VII: BIODATA OF CO- INVESTIGATOR

1. Full Name (in Block Letters) : N CHANDRAKANTH
2. Designation : Scientist - B
3. Department /Institute /University : CSRTI, Berhampore
4. Address for communication : SBG, CSRTI, Berhampore
5. Date of birth : 24/04/1986
6. Sex : Male
7. Education onwards & (Post Graduation onwards & Professional Career)

Name of the university	Degree Passed	Year of Passing	Subjects taken with Specialization	Class/ Divn.
Punjab Technical University, Jalandhar	M. Sc.	2009	Biotechnology	I
University of Mysore, Mysore	Ph.D.	2016	Biotechnology	-

8. Awards: [Not required for house personnel]:

Year	Award	Agency	Purpose	Nature
Nil	Nil	Nil	Nil	Nil

9. Position Held / Research Experience in various institutions:
[Not required for in –house personnel]
10. Memberships/Fellowships: [Not required for in-house personnel] :
11. Patents: [Not required for in-house personnel]:
12. Publications (Number only): 12
Books: 01
Research Papers, Reports: 11
General articles: Ni
13. Project(s) submitted / being pursued / carried out by Investigator:

Sl.No.	Title of the Project/ Program	Funding agency	Duration From and To	No of Scientists /Associates working under the project	Total approved cost of the project (Rs.in lakh)
1	Development of thermotolerant bivoltine breeds / hybrids of silkworm, *Bombyx mori* through marker assisted selection- **AIB 3602**	Central Silk Board	Nov 2016 to April 2021	4	10.55
2	Development of high temperature and high humidity tolerant bivoltine breeds of silkworm, *Bombyx mori* L.- **AIB 3547**	Central Silk Board	June 2015 to June 2017	3	0.33
3	Development of region specific bivoltine silkworm	Central Silk Board	Aug 2011 to Dec	3 scientists from	10.00

	breeds suitable for highly fluctuating and seasonally variable climatic conditions of Eastern and North-Eastern India- **AIB 3466**		2016	CSRTI, Berhampore and Sub unit incharges of 5 stations	
4	Improvement of leaf quality and productivity through external application of seaweed extracts in mulberry (*Morus alba* L.)- **PIN 3587**	Central Silk Board	Oct 2016 to Sep 2017	3	0.40
5	Evaluation of multivoltine germplasm to identify potential parents for developing cross breeds suitable for Southern and Eastern India-**AIB 3577**	Central Silk Board	March 2016 - February 2019	8	Total- 21.20 For Institute – 3.90
6	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-**ARP-3605**	**DBT** funded collaborative project with SBRL, Bengaluru	April 2017 to	8	2.46
7	Maintenance of silkworm germplasm- **BAI(RP)-003**	Central Silk Board	Continuous	4	-

14**.** Highlights of outcome / progress of the project (s) handled during the past 10 years their outcome and utilization (in 200 words).
NIL

PART VII: BIODATA OF CO- INVESTIGATOR

1. NAME : **Shri . N.B Kar**
2. EMPLOYEE NO. : 003322
3. OFFICIAL DESIGNATION : Scientist-D (Reeling & Spinning)
4. PROJECT DESIGNATION : Co-Investigator
5. EXPERTISE AREA : Mechanical Processing of Textiles
6. INSTITUTE NAME : Central Sericultural Research & Training Institute, Berhampore-742 101, West Bengal.
7. INSTITUTE ADDRESS : Central Sericultural Research & Training Institute, Berhampora-742101, West Bengal.
8. TELEPHONE : 03482 – 251046, 251233, 253962
9. TELEX/E-MAIL : karnb@rediffmail.com
10. FAX : 03482 –251046
11. BIRTH YEAR : 1959
12. SEX : Male
13. EDUCATIONAL :

HIGHEST DEGREE (DEGREE ON WARDS	YEAR	UNIVERSITY	COUNTRY	SUBJECT

B. Sc. (Tech) in Textile Technology	1980	Calcutta	India	Spinning, Weaving, Dyeing & Printing, Fibre Science, Testing etc.
M.Sc(Tech) in Mechanical Processing of Textiles	1999	Calcutta	India	Spinning, Weaving, Fibre Science etc.

14. TRAINING UNDERGONE:

COURSE NAME/ SUBJECT	ADDRESS OF INSTITUTE	DURATION	PERIOD		SPONSORED AGENCY
			FROM	TO	
1. Refresher Training on Mulberry	CTR&TI, Ranchi	Three Weeks	1987		
2.Tribal Orientation Training Programme	Tribal Research Institute, Ranchi	One Week	1987		
3.Trainers' Training Programme	CSTRI, Bangalore	Ten days	1992		
4.Entrepreneur Development Programme	NISIET, Hyderabad	Two weeks	1993		
5.Computer Training	CSR&TI, Berhampore	Two weeks	1994		**CSB**
6.Orientation Training	CSTRI, Bangalore	One day	2001		

15. EXPERIENCE:

ORGANISATION/ INSTITUTE	DESIG-NATION	DURA-TI0N	PERIOD		SUBJECT AREA	SIGNIFICANT ACHIEVEMENT
			From	To		
CTR&TI, Ranchi	SRA	4 yrs	1986 (July)	1990 (May)	Research on Tasar Reeling, Spinning & fiber technology	• Design & development of Reeling Cum Twisting machine (1987 – 1990). • Design & development of Studies on a User-Friendly 2-Spindle horizontal Tasar Reeling machine (1988 – 1990). • Studies on the weaving of Tasar raw, dyed & waste spun yarn for product diversification (1988 – 1990).
RSDTC, Fakirpur, Orissa	SRA	1¼ year	1990 (May)	1991 (Aug)	Tasar Research Extension & Training	Associated with Research extension under CTR&TI, Ranchi.
DCTC, Dabok, Rajasthan	SRO	App. 2½ years	1991 (Sept)	1994 (Mar)	Mulberry Research Extension & Training	• Associated with extension activities under NSP • Associated with

						Research extension under CSTRI, Bangalore
CSR&TI, Berhampore	SRO	3½ years	1994 (Mar)	1997 (Dec)	Research on Mulberry Reeling, Spinning & fibre Technology	• Designing of Reeling machine suitable for West Bengal condition to reel N, Nx G & N x Bi cocoons **(1988 – 1994)** • Development of cocoon drying chamber suitable for West Bengal Condition **(1989 – 1994).** • Development of Water Correction Kit for cooking and reeling with reference to the states of eastern region **(1987 – 1994).**
Study Leave	SRO	2 years	1998 (Jan)	1999 (Dec)	Mechanical Processing of Textiles	Obtained M.Sc (Tech) degree from Calcutta University
DCTSC, Madhughat, Malda, West Bengal.	SRO	4¼ years	2000 (Jan)	2004 (Apr)	Mulberry Research Extension & Training	Associated with Research extension under CSTRI, Bangalore.
CSR&TI, Berhampore	SRO	App. 4 years	2004 (May)	2006 (August)	Research on Mulberry Reeling, Spinning & fibre Technology	• Associated with 4 no. Ongoing & 5 no. Concluded Research Projects as CI. • Associated with 2 no. Ongoing & 4 no. Concluded Research Projects as non-CI. • Associated with 03 no. Ongoing & 4 no. Concluded Research Programmes.

CSR&TI, Berhampore	Scientist - C	App. 8 years	2006 (Aug)	2014 (Feb)	Research on Mulberry Reeling, Spinning & fibre Technology	
CSR&TI, Berhampore	Scientist - D	App. 2 years	2014 (Feb)	Till date	Research on Mulberry Reeling, Spinning & fibre Technology	

16. ACTIVCITY **:**

a) PRESENT DISCIPLINE OF WORK **: Reeling & spinning**

b) PRESENT AREA OF WORK **: Post Cocoon Technology**

17. TIME ALLOCATION [IN %] & COST **:**

[NOTE:One week = 2%] [For one year]

SL. NO.	NATURE OF WORK	TIME %	NO. OF WEEKS	COST [RS. IN LAKHS]	COST [RS. IN LAKHS]

18. PROJECTS PURSUED:

A] PROJECTS UNDER PROGRESS

SL. NO.	PROJECT CODE	PROJECT TITLE	RESEARCH TIME SPENT [%]	COST [RS.]
1	BAI(P) - 014	Studies on the Reelability of Multivoltine Hybrid Cocoons during adverse climatic condition in Eastern and Northeastern Region	50%	0.024
2	AIB - 3480	Development of Silkworm (Bombyx mori *L*) Breeds from a Gene Pool with Higher Genetic Plasticity	9%	0.024
3	AIB - 3466:	Development of Region Specific Bivoltine Breeds suitable for Highly Fluctuating Seasonally Variable Climatic Condition of Eastern & North-Eastern India	9%	0.024
4	Other Programmes		32%	0.024
5				0.172
		TOTAL	**100 ***	

B] COMPLETED PROJECTS

APR 3250: Development of Rearing Package for optimizing cocoon yield in West Bengal

APS 3238: Induction of Trimoulting in Bivoltine silkworm by physiologically active chemicals and their utilization as male parents for multi x bi hybrid production.

AIG 002: Utilization of Syngenic Lines for Improvement of Shell Weight & Survivals in *Bombyx mori-L*

PPA 3249: Effect of Integrated Plant Nutrition Systems on Mulberry Crop Production &

Protection

PPA 3223 Updation of Improved Package of Practices for newly Authorized Mulberry Varieties under Irrigated Condition

AIB 3237 Utilization of Polyvoltine Breeds for Improvement of Survivals in Bivoltine Silkworms *Bombyx Mori*-L.

AIB 3291 Evaluation of viable Sex Limited Bivoltine Breeds of *Bombyx Mori-L.*

PPA 3358 Organic Farming in Mulberry-An Approach for Improvement of Silk Industry.

PPA 3366 Development of Integrated Package for Raising Chawki Leaves & Young Age Silkworm Rearing for Successful Cocoon Crops

Other Projects & Programmes:

***** = 100% IS THE TOTAL RESEARCH TIME WHICH IS EQUIVALENT TO THE % OF THE TIME SPENT ON RESEARCH AS INDICATED UNDER 'RESEARCH' AGAINST POINT NO. 17.

19. **Highlight of the outcome/ progress of the project (s) handled during the past 10 years, their outcome and utilization (in 200 words):**

The findings of the various research projects from the different sections particularly Silkworm Breeding and Genetics Section and Silkworm Physiology and RTI Section are finally substantiated by the post-cocoon assessment. Satisfaction of a reeler has the ultimate role for acceptance of a technology developed either by SBG or RTI. Part contribution of a reeler acts behind the success of a breeder. Previously eastern part of India reared only Nistari and Nx Bi dfls in 3-5 seasons according to the meteorological area. Now after introduction of various high yielding silkworm breeds, both multi x multi and multi & bivoltine the productivity of the farmers per unit laying and the rearing capacity of the farmers have been increased which also enable them to fetch higher income. A suitable alternative to Bivoltine component NB4D2 has been found and as such SK6 x SK7 (FC) is going to replace NB4D2 successfully that can be reared throughout the year irrespective of climatic vagaries. Extensive study on breed development has also pave the way for some other combinations of silkworm races viz.; M.Con.1 x M.Con.4, Nistari x M.Con.4, M.Con.1 x B. Con.4 and M.Con.4 x B.Con.4 to replace the existing low productive breeds / hybrids. I was actively associated as co-investigator with all the projects during last seven years formulated by SBG or RTI section. Some work on formulation of an ideal package of practices of rearing during different climatic conditions has also come out with definite recommendation. Large scale testing of breed / hybrid at farm level, Cluster Promotion Programme at different zones has supported the breeds to become popular at commercial level.

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 through out the duration of the project.
j. The Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Executive Authority
Institute with Seal
Date:

1. Signature of Principal Investigator
Date:

2. Signature of Co-Investigator-1
Date:

3. Signature of Co- Investigator -2
Date: