AIB 3547

PROJECT

Development of high temperature and high humidity tolerant bivoltine breeds of silkworm (*Bombyx mori* L.)

Duration: **July 2015 to June 2017**

Budget : **0.33 lakh**

Principal Investigator : Dr. N. Suresh Kumar

Project Co-ordinator : Dr. A. K. Saha

CENTRAL SERICULTURAL RESEARCH & TRAINING INSTITUTE

Central Silk Board : Ministry of Textiles : Govt of India

Berhampore - 742101, West Bengal

:

PROFORMA FOR COLLECTION OF DATA OF RESEARCH PROJECTS IN SERICULTURE

PART-I : GENERAL INFORMATION

1.	**Name of the Institute / University / Organization submitting the Project Proposal**	:Central Sericultural Research and Training Institute, Berhampore, West Bengal
2.	**Status of the Institute (s)**	: N.A.
3.	**Name (s) and designation(s) of the Executive Authority of the institute / University forwarding the application**	: Dr.A.K.Saha, Director(I/C)
4.	**Project Title**	: Development of high temperature and high humidity tolerant bivoltine breeds of silkworm (*Bombyx mori* L.)
5.	**Category of the Project**	: Animal (A)
6.	**Specific Area**	: Silkworm Improvement
7.	**Duration**	**:** July 2015 to June 2018
8.	**Total Cost**	**: 0.33** lakhs
9.	**Is the Project single Institutional or multi-institutional**	**:**
10.	**If the Project is multi-institutional, please furnish the following : Name , Designation and Address of the Project Co-Ordinator.**	**: N.A.**

11. (a) Project Summary:

To initiate the breeding programme initial silkworm parents will be collected from different breeding centres. Screening of silkworm breeds reported so far as thermo-tolerant or tolerant to adverse climatic situation based on last 3 years performance *i.e.*, July-August and September collected from authentic sources. About 10 breeds from bivoltine and 5 breeds from multivoltine germplasm stock of CSR&TI, Berhampore and 12 breeds of other authentic source will be selected. The silkworm breeds will be reared during highly adverse climatic situations in North and North Eastern zone specially considering the West Bengal conditions during September P_1 rearing when temperature and RH are more than 33ºC and 93% respectively. Based on the performance, 2-4 silkworm breeds showing higher temperature tolerance will be considered for the next experiment entitled ‘induction of thermal stress to development of thermo-tolerant breed’. Thereafter, to improve the survival percentage more than 80%, the survived silkworm larvae from the LD_{50} dose will be maintained under normal condition and the laying prepared from the emerged male and female moths. Again the brushed larvae will be exposed under same temperature and relative humidity for 3-4 generations or as per need in a similar fashion. After achieving the survival more than 85% in a specific stage, the larva will be considered for the next stage to determine LD_{50} dose at temperature >35 ºC and period of survival in the specific stage. As explained earlier, the process will be continued until survival above 80% up to spinning stage (from brushing to spinning) is achieved. The bivoltine breed having the good qualitative and quantitative characters will be crossed with the developed thermo-tolerant breed through introgression method as suggested by Chattopadhyay *et al*. (2000) of Congenic Breeding approach. The developed thermo-tolerant breeds will be subjected for hybrid evaluation under high temperature and high humidity conditions and based on overall performance with special emphasis on survival rate, suitable hybrids will be selected for commercialization.

11. (b) Aims and Objectives:.

The main aim and objective of the project is to develop bivoltine breeds tolerant to high temperature and high humidity which have genetic plasticity to buffer against adverse climatic conditions of West Bengal

PART-II: PARTICULARS OF INVESTIGATORS

12. a) Name: : Dr.N.Suresh Kumar

Date of Birth : 01-06-1956
Sex: : Male
Indicate whether Principal Investigator/ Co-investigator : Principal Investigator
Designation : Scientist-D
Department : Silkworm Breeding and Genetics,
Institute/University: Address : CSRTI, Berhampore

12.b) Name: : Dr.A.K.Verma

Date of Birth : 28.12.1960.

Sex: : Male
Indicate whether Principal Investigator/ Co-investigator : Co-investigator
Designation : Scientist-D
Department : Silkworm Breeding and Genetics,
Institute/University Address : CSRTI, Berhampore

13. No. of Projects being handled by Each investigator at present

Principal Investigator : PI in 2 projects and one Programme CI in 2 Programmes
Co-investigator : PI in 1 project and 2 new programmes

14. Proposed Research Fellows: One . [Detailed justification with work sharing is a must]

As both the PI and CI are not having the expertise in carrying out the biochemical analysis the services of the JRF will be utilized for the work. Besides the JRF will be assisting the rearing and breeding work of the project. Hence, one JRF is essential for the project

PART-III: TECHNICAL DETAILS OF THE PROJECT

15. **Introduction**

The Indian sericulture industry is beset with many problems. One of the main problems is the inability to produce quality silk of international grade. The quality silk can be produced only form bivoltines. The bulk of silk produced in India is from Multivoltines which are of inferior quality. Therefore, it is highly pertinent to have more productive bivoltine silkworm breeds capable of producing quality silk. However, the hot climatic conditions of India is not conducive to rear productive bivoltines. Hence, there is an urgent need to develop bivoltine breeds which can yield stable crops under the adverse climatic conditions. Accordingly, efforts should be focused by the silkworm breeders to develop bivoltine breeds with genetic plasticity to buffer against the adverse climatic conditions. The main constraint of the tropical environment is the high temperature coupled with high and low humidity. It is a well established fact that the bivoltines are highly vulnerable to high temperature coupled with high and low humidity especially in the late instars. The hot climatic conditions of tropics prevailing particularly in summer are contributing to the poor performance of the bivoltine breeds and the most important aspect is that many quantitative characters such as viability and cocoon traits decline sharply when temperature is high. The silkworm breeds developed for tropical conditions in India have to adapt to both seasonal and local conditions for stable cocoon production under the high temperature associated biotic and abiotic conditions. In India, mulberry leaves are available throughout the year including the summer months. However, during summer the rearing of bivoltines are very difficult with frequent crop losses. Therefore, the farmers are forced to switch over to multivoltine x bivoltine hybrids which are comparatively more stable under such environmental conditions. Since, the rearing of bivoltines in summer months becomes very difficult, the concept of bivoltine throughout the year to produce quality silk becomes jeopardized. In India, the adverse climatic conditions during summer is not the same throughout and some location having high temperature coupled with high humidity and in some high temperature with low humidity besides, poor leaf quality at times. Summer breeds are having significant importance in increasing cocoon production through rearing bivoltine hybrids round the year in tropical areas. The advantages of summer hybrids are high pupation rate, adaptabilities to high temperature coupled with high and low humidity and inferior food quality during the rearing. Stable cocoon crop under the bad conditions of high temperature with low quality mulberry leaves are difficult, but summer breeds/hybrids should have the potentiality for increasing production under such un-favourable weather conditions.

Temperature is one of the factors that can influence the diversification of life with the interaction of other environmental components like humidity, light etc. Global climate change is occurring. Mean temperatures are predicted to increase 1.5 – 3.5°C by 2040. Being piokilothermic animals, development in insects is strongly influenced by external temperature. If a substantial change in temperature and humidity takes place, the ability of the organism to survive largely depends on its genetic architecture in the gene pool which may help to synthesize immuno protein against this change. Under extreme temperature, cells become apoptic because of denaturation of proteins and enzymes, which leads to altered membrane structure and function. Accumulation of denatured protein triggers production of heat shock protein, which assists in protein folding. It has been reported that silkworms can grow in the temperature range of 10 – 32°C, but their physiological activities are reduced when their body temperature falls below 15°C or exceeds 30°C, resulting in their poor performance. Silkworm is a poikilothermic animal. From hatching to spinning period there is a need for specific environment such as 27 – 28°C temperature with 85% Relative Humidity from hatching to III stage and for IV to V stage and spinning it needs 25 – 26°C and 75% Relative Humidity. Variation in temperature above 28°C and humidity above then 80% causes physiological deficiency resulting in the reduction of cocoon production. Therefore, development of suitable breeds for rearing under high temperature and high humidity conditions always been perceived as an extremely challenging task. As such, it is essential that the breeding strategies need to be directed towards the development of sustainable thermo-tolerant bivoltine breeds to produce quality raw silk to improve the unit production under tropical climate. Generally multivoltine breed produces low quality silk but bivoltine produces good quality silk at congenial environment but in tropical condition when temperature and humidity are high and highly variable, the survival of bivoltine breed comes down very low to nil.

In general, introgression of the hardiness character from the tropical multivoltine strain/breed into the temperate bivoltine strain/breed offers promising breeding material having good yield parameter and quality character where survival rate of silkworms is considered as the main criteria for robustness. Accordingly, various attempts were made in the past by different sericulture research institutes to develop a robust bivoltine breed by crossing with the polyvoltine and bivoltine breed through conventional breeding approach and used for hybridization. However, the attempts were failed to reach to the target due to adoption of directional selection, *i.e.,* development of Recombinant Inbreeding Line (RIL) without considering the genetic basis for hardiness and also for other characters of silkworm which is very difficult to introgress by single crossing in other breed as maximum qualitative and quantitative characters are controlled by multiple genes. So, without adoption of introgression method for multiple genes it is difficult to fulfill the target to develop a hardy/robust bivoltine. As such in tropical country, as a first step a target need to develop a thermo-tolerant breed *i.e.,* 35 ± 1°C and RH 90% with survival percentage >80% and thereafter introgression could be made as second step to the bivoltine that can be reared during adverse season (temperature-35°C and Relative Humidity of >90%). Exposure to high temperature is lethal for any living organism and the rate of lethality depends upon the exposure period and the magnitude of the temperature. As silkworms are poikilothermic and thrive best within a temperature range of 23-28°C, fluctuation of temperature affects the normal physiology, survival and pupation. The tropical Indian multivoltine breeds of *Bombyx mori* L. (Pure Mysore, C. Nichi and Nistari) are more tolerant to high temperatures, as against the exotic bivoltine breeds of temperate origin. India being a tropical country, temperature shoots up in day time. In summer it goes up to 37 to 40°C or even more. These fluctuations in temperature have an adverse effect on the survival and pupation of silkworms especially the bivoltine breeds incurring heavy loss to the industry. As a means to overcome this loss due to the adverse effect of the 'heat-shock' being faced regularly, there is a need to develop thermo-tolerant bivoltine breeds.

In this direction, an attempt is to be made to determine the LD_{50} under thermal stress in silkworm and identifying the critical temperature to keep under exposure. In the proposed project, it is envisaged to carry out the work after determination of LD_{50} by keeping the fixed humidity at above 85%, exposing the larvae in different stages for a period to identify specific time period for a particular stage having the mortality 50%. Thereafter, attempts will be made to improve the survival percentage to more than 80 - 85% by exposing the larvae continuously in the same temperature for 4 to 5 generations or as per need. Then, the same line will be exposed to high temperature in the next stage of development to overcome LD_{50} at this stage followed by exposing the larvae continuously in the same temperature. The induction of thermal stress will be further continued until breeds with high temperature (35°C ± 1°C) and high humidity (80 ± 5%) tolerance are to be obtained up to final stage, *i.e.*, tolerant to high temperature and high humidity conditions from brushing to spinning (proposed method is enclosed in annexure – I).

The varied climatic conditions of North East which are hot and highly humid particularly during summer (June to August having 35-36oC and 90-98% relative humidity together with rainfall that every time cause the failure of cocoon crops during the period) make it different from rest of the country. The existing hybrids are unable to satisfy the demands of the local farmers. The proposed method of work is the first attempt at the CSRTI, Berhampore to evolve high temperature tolerant bivoltine breeds for P_1 rearing and their use as male component for promising multivoltine × bivoltine hybrids under target agro-climatic conditions. Realizing the urgent need to develop breeds that can withstand high temperature and high humidity conditions in India, this project has been proposed.

15.1 **Definition of the Problem**

(a) Origin of the project

Indian sericulture industry is multivoltine oriented and hence the quality of silk is of low grade. Quality silk can be produced only through bivoltines.. However, the hot climatic conditions prevailing in India is not conducive to rear the bivoltines already available. Therefore, there is an urgent need to develop high temperature and high humidity tolerant bivoltine breeds which can withstand the adverse climatic conditions of the tropics.

b) Expected outcome

The successful completion of the project is expected to come out with the development of thermotolerant bivoltine breeds with genetic plasticity to buffer against the adverse climatic conditions.

15.2 **Origin of the Proposal / Rationale of the Study**

Eastern India, especially the state of West Bengal experiences extreme variation in temperature, relative humidity and rainfall. According to climatic conditions, the commercial seasons are broadly divided into two, favourable and unfavourable. During unfavourable season, because of prevalence of high temperature and humidity as well as rainfall, most of the rearers rear indigenous multivoltine breed, Nistari during the period, which is low productive. Keeping the prevalence of variable climatic condition in mind and realizing the importance of season specific bivoltine hybrids as well as advantage of rearing of F1 hybrids during different commercial season, bivoltine breeds will be developed and their hybrids will be reared in three commercial seasons with adverse climate for two years. From these hybrids, selection of season specific better performing multivoltine hybrids will be made.

15.3 **Relevance to the current issues and expected outcome**

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. The climatic situation of West Bengal is broadly categorized into two i.e., the favourable (November to March) and unfavourable (May to September). It has been observed that bivoltine P_1 rearing to prepare multi x bi hybrid dfls for three commercial crop (June-July, August-September and November-December) is not successful as the P_1 bivoltine rearing to prepare multi x bi layings for aforesaid commercial seasons fall under unfavourable season [high temperature (>35^0 C) and high humidity (>85-99 %] which are not congenial for bivoltine silkworm rearing. Therefore, farmers are forced to restrict their rearing only with Nistari, the indigenous multivoltine strain having horizontal tolerant potentiality both at P_1 and in commercial level during the adverse month. Now a day's multivoltine hybrid of Nistari is being widely reared at commercial level in West Bengal during adverse seasons though the production.

15.4 **Objective**

To develop thermotolerant bivoltine breeds with genetic plasticity to buffer against the adverse climatic conditions of West Bengal

16. **Review of status of Research and Development on the subject.**

16.1 **International Status**

Rapusas and Gabriel (1976) reported that environmental factors especially temperature and humidity play a very important role in the life cycle of silkworm in determining the cocoon characters and its existence in a particular zone. The environment is dynamic and different environment brings about profound changes in the physical and biotic factors governing the expression of commercial characters in silkworm (Kobayashi *et al.,* 1986). Hsieh *et al.* (1995) studied the thermo-tolerance of silkworm and concluded that high temperature (36±1^{o}C) affects on the survival more than other characters and the multivoltine breeds are more tolerant to high temperature than bivoltine breeds during 4th, 5th instars and pupal stage. In comparison to other insects, the threshold temperature that induces 100% mortality in silkworm at 46 ^{0}C for Chinese and Japanese breeds (Hsieh *et al.,* 1995) and Indian Pure Mysore (Lohmann and Riddiford, 1992).

The poor performance of the bivoltine strains under tropical conditions with respect to viability and cocoon traits decline sharply when temperature increases above 28^{0}C (Shibukawa,1965). Ueda and Lizuka (1962) reported silkworms are more sensitive to high temperature during 4th and 5th instars. Shibukawa (1965) studied the viability of silkworm for 19 generations at two temperatures, 28^{0}C (high) and 90% RH and 25^{0}C (normal) and 80% RH and showed that the line selected at high temperature and humidity performed better than the line selected at normal temperature (Evgenev *et al*., 1987; Coulon- Bublex and Mathelin, 1991; Wu and Hou, 1993; Zhao *et al*., 2007; Moghaddam *et al*., 2008).

Huang et *al*. (1979), He and Oshiki (1984) suggested that survival rate of silkworm as a main criterion for evaluating thermo-tolerance. Tazima and Ohnuma (1995) while synthesizing high temperature resistant silkworm breeds confirmed the genetic nature of thermo tolerance by selection based on pupation rate of silkworm reared under high temperature conditions in 5th instar. Kato *et al*. (1989) subjected silkworm larvae to 25, 32 and 36^{o}C for early and last three days of 5th instar. Sensitivity to high temperature was found more pronounced at 36^{o}C of last three days. Shirota (1992) attempted to develop temperature resistant breed from the Japanese strain “NK” based on pupation rate reared under high temperatures confirmed that high temperature resistant character was dominant which confirmed the view of Kato *et al*. (1989). In recent, effect of temperature on the development and survival of the Argentine ant also have been reveled by Abril *et al.* (2010).

Shao *et al.* (1987) developed the silkworm hybrids “Xinhang” and “Keming” for summer rearing in China by crossing polyvoltine race with productive bivoltine race and subjecting for temperature treatment of 29-32^{o}C and humidity of 85%. Burdon (1987) opined that heat stress to animal cells is the vigorous but transient activation of a small number of specific genes, previously either silent, or active at low levels. . The limits of tolerance are not fixed. Indeed it has been known for some time that exposure to a near lethal temperature often leads to a degree of adaptation so that a previously lethal temperature is tolerated (Craig, 1985;

Lindquist, 1986; Mosley, 1997; Bijlsma and Loeschecke, 1997). So, determination of lethal temperature is important for silkworm to find out thermo-tolerant capacity.

16.2 **National Status**

Eastern India, especially the state of West Bengal experiences extreme variation in temperature, relative humidity and rainfall. According to climatic conditions, the commercial seasons are broadly divided into two, favourable and unfavourable. The former falls between October to March, when the climatic conditions are congenial for silkworm rearing. Autumn (November) and Spring (February) crops comes during this period. April (Baisakhi), commercial crop is also considered as partially congenial for silkworm rearing in terms of prevalence of low humidity (Krishnaswami *et al.*, 1971; Moorthy and Das, 2007). On the other hand, the unfavourable period starts from May to September and not conducible for silkworm rearing, since temperature and humidity are high. June-July (Shravani) and August -September (Badhuri and Aswina) crops are conducted during this period. Because of prevalence of high temperature and humidity as well as rainfall, most of the rearers rear indigenous breed, Nistari during the period, which is low productive. But multi x bi hybrid can be successfully reared during autumn and spring seasons of the plains, which could increase the silk production (Sengupta, 1987). F_1 hybrids are superior to parental strains in terms of higher tolerance to disease, higher adaptability to unfavourable environmental situation and produce uniform and stable crops due to hybrid vigour. But the major problem is the rearing of parent silkworm during seed crop, because most of the seed crops fall during unfavourable season, when temperature as well as humidity remains high. Conducting seed crop for Autumn (Agrahayani) commercial crop is very much difficult, because of prevalence of high temperature and high humidity during the period (September-October). The unsuccessful rearing of bivoltine parent rearing leads to unsuccessful production of multi x bi eggs. Reddy *et al.* (2002) observed that the pupation rate in Indian popular bivoltine breed, NB_4D_2 is significantly influenced by both low and high humidity.

To overcome such aforesaid environmental problem, the Central Sericulture Research and Training Institute, Berhampore identified few silkworm hybrids according to requirement of the region such as, M_{12}(W) x M_6M_{81}, M_{12}(W) x M_6DP(C) as V_3 x V_3 and two three way crosses *like* M_{12}(W) x (SK_6 x SK_7), M6DP(C) x (SK_6 x SK_7). Vijaya Kumari *et al*. (2001) observed that at high temperature (35 ^{0}C) and low humidity (50±5%) and high humidity (85±5%) conditions, pupation rate was drastically reduced in productive hybrid, CSR_2 x CSR_5.

Suresh Kumar *et al.* (1999) indicated that the deleterious effect of high temperature and high humidity was more pronounced in productive bivoltine hybrids than the robust bivoltine hybrids. Pupation rate of those hybrids are more where the female parents used are more tolerant under high temperature and high humidity conditions (Suresh Kumar *et al.*, 2005). Higher survival of the hybrids than the pure races under high temperature conditions are reported by Suresh Kumar *et al.* (1999, 2001, 2005), Suresh Kumar and Yamamoto (1995). Suresh Kumar *et al.* (2004, 2006) developed CSR_{18} x CSR_{19} and CSR_{46} x CSR_{47} by subjecting the silkworms at 36±1 ^{0}C and 85±5% RH from third day of 5^{th} instar.

In India, few tropical indigenous strain/breeds (v3) are well adapted to fluctuating tropical climatic conditions characterized by high temperature, but they are poor in productivity. When both the parental strains and hybrids are raised in unfavorable environmental conditions, performance of hybrids will be much superior to both the parental strains (Nagaraju *et al.,* 1996). Sudhakara Rao *et al*. (2002) made an attempt to select suitable breeding resource materials to develop silkworm breeds by giving high temperature (36±1°C) and low humidity (65±5%) stress in V instar for three days during the course of breeding to suit the locations with high temperature and low humidity situations and raised two bivoltine hybrids CSR_2 x SR_5 and SR_1 x SR_4. Silkworm breeding efforts at Karnataka State Sericulture Research and Development Institute (KSSRDI) also developed the hybrid KSO_1 x NP_2 (Krishna Rao *et al*., 2001). One high temperature tolerant bivoltine hybrid $APSHTO_5$ x $APSHTP_2$ developed by giving thermal stress from brushing to spinning at 32°C & 50% RH by Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI) was recommended for commercial utilization in Andhra Pradesh during summer season (Raju, 2010). Pupation rate is considered as the main yard stick to measure the tolerance of silkworm breeds under high temperature environment. Accordingly, the breeds with pupation rate above 60% at 36±1 ^{0}C and 85±5% RH are considered as temperature tolerant and below 25% are considered temperature sensitive (Suresh Kumar *et al*., 2005).

Pillai and Krishnaswami (1980, 1987) reported the effect of exposure of silkworm larvae to high temperature in the 5^{th} instar and concluded that the low survival rate was due to the low feeding activity of the silkworm resulting in the physiological imbalance and poor health of the larvae. They reported that exposure to high temperature during the later developmental stages considerably reduce the survival rate, cocoon quality and fecundity of the breeds. Vijaya Kumari *et al*. (2001) observed that at high temperature (35 ^{0}C) and low humidity (50±5%) and high humidity (85±5%) conditions, pupation rate was drastically reduced in productive hybrid, CSR_2 x CSR_5. Reddy *et al.* (2002) observed that the pupation rate in Indian popular bivoltine breed, NB_4D_2 is significantly influenced by both low and high humidity.

In India, few tropical indigenous strain/breeds (v3) are well adapted to fluctuating tropical climatic conditions characterized by high temperature, but they are poor in productivity. When both the parental strains and hybrids are raised in unfavorable environmental conditions, performance of hybrids will be much superior to both the parental strains (Nagaraju *et al.* 1996). Suresh Kumar *et al.* (1999) indicated that the deleterious effect of high temperature and high humidity was more pronounced in productive bivoltine hybrids than the robust bivoltine hybrids. Pupation rate of those hybrids are more where the female parents used are more tolerant under high temperature and high humidity conditions (Suresh Kumar *et al*. 2005). Higher survival of the hybrids than the pure races under high temperature conditions are reported by Suresh Kumar *et al.* (1999, 2001, 2005), Suresh Kumar and Yamamoto (1995), Suresh Kumar *et al*. (2004, 2006) developed CSR_{18} x CSR_{19} and CSR_{46} x CSR_{47} by subjecting the silkworms at 36±1 ^{0}C and 85±5% RH from third day of 5^{th} instar.

16.3 **Importance of the proposed project in the context of current status**

Now a day's multivoltine hybrid is being widely reared at commercial level in West Bengal during adverse seasons. To solve this problem, development of temperature tolerant region and season specific bivoltine hybrids is highly required at present situation.

It is a challenge to overcome the problem by raising a sustainable high temperature tolerant bivoltine breeds with genetic plasticity to buffer against the adverse seasons (June, August and September) in West Bengal. Such breeds are urgently required to increase the P_1 bivoltine cocoon production for the production of multivoltine hybrids and also bivoltine hybrids to increase the productivity as well as quality of silk.

In India, tropical climate prevails in major part of the sericulture belt, where temperature goes beyond the ambient during summer causing adverse effect in the silkworm rearing. Silkworm rearing is practiced five times taking advantage of the availability of abundant mulberry leaves due to high rainfall and fertility of soil. But, the practice of rearing of silkworm hybrids is very much restricted due to highly fluctuating climatic conditions. The favourable climate for silkworm rearing is from November to March and unfavourable is from April to September. It has been observed that raising of male bivoltine parent (P_1) for multi x bi hybrid seed production for three commercial crops during June–July, August-September and November–December seasons was not successful owing to its rearing during unfavourable season characterized by high temperature (>35ºC) and high humidity (85-99 %) conditions. On the other hand, bivoltine P_1 seed crop rearing in September for preparing multi x bi hybrid for Autumn (November) commercial rearing falls under very harsh climatic condition (Das *et al.,* 2005a, b, 2006). Hence, there is an urgent need to develop high temperature (35ºC ± 1ºC) and high humidity (RH, 80±5%) tolerant bivoltine breeds that can sustain wide fluctuating environmental conditions and can be used as a male parent for cross breed production so as to make sericulture a sustainable avocation in the region.

16.4 **Anticipated Products, processes/Technology, Packages/ Information or other outcome from the project and their expected utility**

The successful completion of the project will lead to the development of robust bivoltine breeds/hybrids suitable to the West Bengal Conditions and the bivoltine pure breeds can be reared in adverse seasons and can be effectively utilized for the production of multivoltine x bivoltine hybrids throughout the year without any difficulty.

16.5 **Expertise available with proposed investigation group/institution on the subject of the project**

Name of the Scientists	**Designation**	**Experience**
Dr.N.Suresh Kumar	Scientist-D	More than 28 years of experience in silkworm breeding
Dr.A.K.Verma	Scientist-D	More than 22 years of experience in silkworm breeding

16.6 **List of Five Experts in India in Proposed Subject Area**

Sl. No.	**Name**	**Designation**	**Address**
1	Dr.H.K.Basavarja	Scientist-E (Retired)	APSSRDI, Hindupur
2	Dr.S.Krishna Rao	Scientist-E	KSSRDI, Bangalore
3	Dr.P.J.Raju	Director	APSSRDI, Hindupur
4	Dr.R.K.Datta	Director(Retired)	Silver Oak, Srirampura-II stage, Mysore
5	Dr.G.Subramanya	Professor	University of Mysore, Mysore

17. **Work Plan**
17.1 **Methodology**

B. EXPERIMENT CODE: E02

1. Experiment title: Induction of thermal stress for the development of thermo-tolerant breed and biochemical characterization.

2. Objectives: For the determination of LD_{50} under thermal stress with constant relative humidity 85 ± 5%, the silkworm larvae exposed continuously at specific stages to find out the temperature (>35 ºC) and the period of survival in a specific stage having more than 50% survival.

3. Materials: Two selected silkworm breeds from previous experiment.

4. **Methodology:** Selected breeds will be brought under biochemical characterization. Thereafter, to improve the survival percentage more than 80%, the survived silkworm larvae from the LD_{50} dose will be maintained under normal condition and the laying prepared from the emerged male and female moths. Again the brushed larvae will be exposed under same temperature and relative humidity for 3-4 generations or as per need in a similar fashion. After achieving

the survival more than 85% in a specific stage, the larva will be considered for the next stage to determine LD_{50} dose at temperature >35 ºC and period of survival in the specific stage. As explained earlier, the process will be continued until survival above 80% up to spinning stage (from brushing to spinning) is achieved as illustrated in Annexure – I.

Annexure – I

Method of induction of thermal stress to improve survival above 85% at stage after stage over LD_{50}:

(Modified proposed Method over Chinese Method)

Determination of LD_{50} and period of exposure under thermal stress at Ist stage with constant Relative Humidity (RH %).

↓

Alive silkworm larvae after LD_{50} will be reared there after at congenial environment up to the time of layings preparation for next generation.

↓

Hatched larvae from the prepared laying will be reared again continuously in earlier temperature (exposure ± 1ºC) with constant RH% for a period determined for this specific stage and afterwards silkworms are taken back to rear at congenial environment up to layings preparation for next generation.

↓

Same procedure mentioned above will be followed for 3 to 4 generations or as per need and to try to improve survival % more than 80 – 85% in a constant RH% (critical temperature may change depending on survival %). These larvae are ready to expose under thermal stress for 2nd stage with constant RH% to determine LD_{50} and period of exposure during this stage (as compared to only normal LD_{50} and exposure) which was already determined at different stage of development) before starting induction experiment.

↓

Similar procedure will be followed like 1st stage for 3 to 4 generation or as per need and try to improve survival % more than 80-85 % in a constant RH% for IInd stage (critical temperature may be vary/change depend on survival %).

↓

Same procedure will be adopted for the remaining each stage for 3 to 4 generations or as per need to improve survival % more than 80 – 85% in the same humidity and induction of thermal stress will be continued until exposed line is capable to with stand 35°C±1°C with constant humidity 80 ± 5% throughout the larval period (critical temperature may be changed depend on survival %).

↓

Fixation of silkworm breeds with thermo-tolerance

C. EXPERIMENT CODE: E03

1. Experiment title: Introgression of qualitative traits in developed high temperature and high humidity tolerant breed.

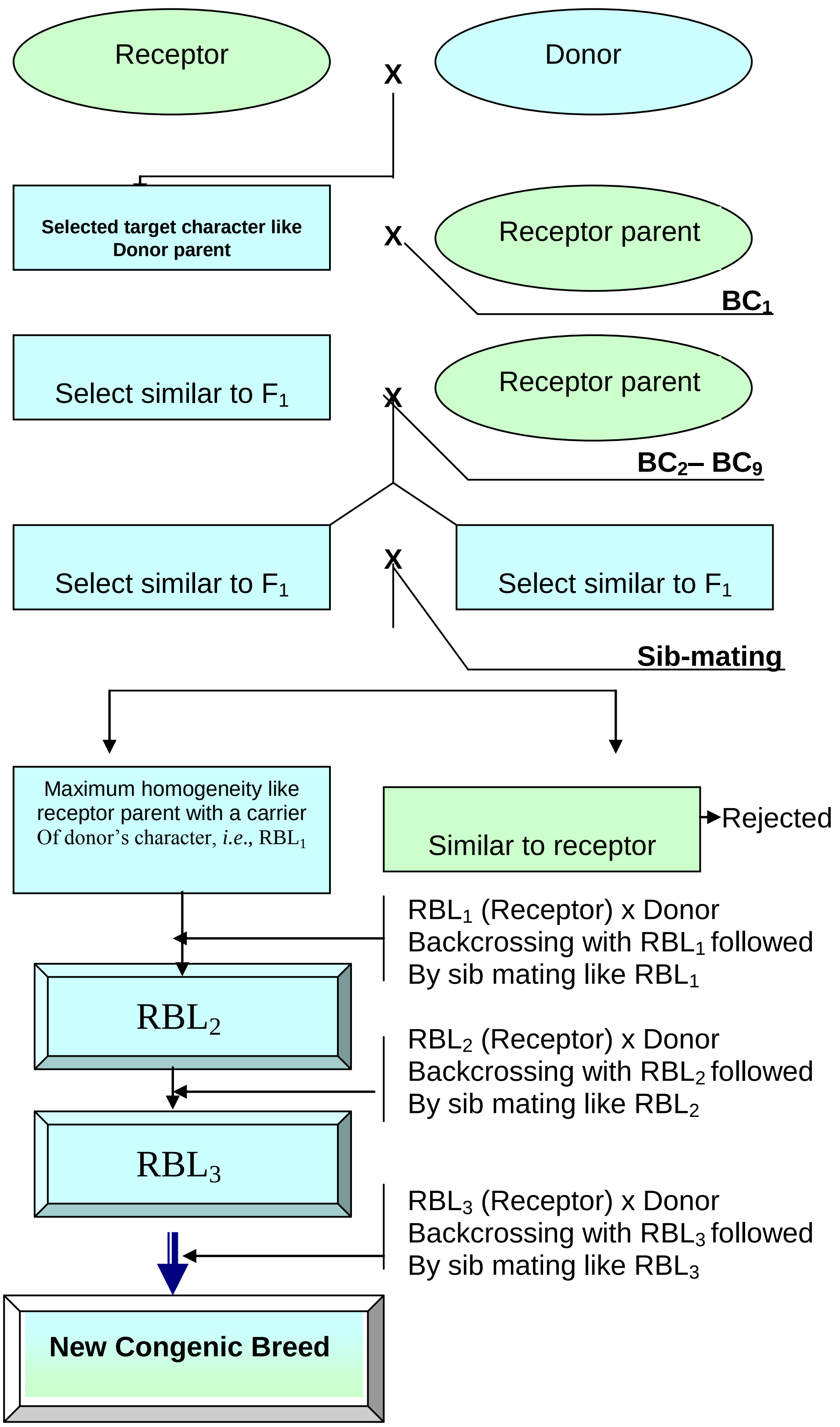
2. Objectives: Development of thermo-tolerant silkworm breed with superior qualitative and quantitative traits with higher survival of >85%.

3. Materials: Productive bivoltine silkworm breeds and thermo-tolerant breeds.

4. **Methodology:** The bivoltine breed having the good qualitative and quantitative characters will be crossed with the developed thermo-tolerant breed through introgression method as suggested by Chattopadhyay *et al*. (2000) of Congenic Breeding approach. The method is schematically enclosed illustrated in Annexure – II.

Annexure II

Scheme for development of Congenic Breeding Approach



17.2 **Organization of Work Elements**:

Name of Scientists	Designation	Time	Organization of work elements.
Dr.N.Suresh Kumar	Scientist-D	30%	Planning, execution, monitoring the project
Dr.A.K.Verma	Scientist-D	15 %	Assisting in the execution of the project

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

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

17.5 **Time Schedule of activities giving milestones**:

Sl.No.	**Organization of work/Milestone /Activity**	**Expected Date of**	
		Starting	**Completion**
1.	Induction of thermal stress and development of thermo-tolerant breed	July 2015	June 2016
2.	Introgression of qualitative traits in high temperature tolerant breed.	July 2016	December 2016
3.	Hybrid evaluation	January 2017	December 2017
4.	Testing of selected hybrids in the field	January 2018	June 2018

17.6 **Project Implementing Agency /Agencies** :

Name of the agency	Address of the agency	Proposed Research Aspects	Proposed Amount	Cost Sharing %
CSB	CSB, Bangalore Pin-560068		0.33 lakhs	100 %

PART-IV: **BUDGET PARTICULARS**

18. **BUDGET** (in Lakhs) : [In case of multi-institutional projects, the budget details should be provided separately for each of the Institute]

A) Non-Recurring (e.g.equipments, accessories, etc.)

Sl No.	**Item**	**1st Year 2015**	**2nd Year 2016**	**3rd Year 2017**	**Total**
A] LAND					
B] BUILDING					
C] VEHICLE					
D] EQUIPMENTS 1.					
E] FURNITURE					
F] FAN & FIXTURES					

G] TOOLS,PLANT & MACHINERIES				
TOTAL				

** EXCLUSIVELY PROCURED/BOOKED FOR THE PROJECT, IF ANY

B) Recurring

B.1. *Manpower* : N.A.

Sl. No	**Position**	**No.**	**Consolidated Emoluments**	**1st Year**	**2nd Year**	**3rd Year**	**Total In lakhs**
	Sub Total B1:						

B.2. Consumables :

S.No.	**Item**	**1st Year**	**2nd Year**	**3rd Year**	**Total**
1.	Paraffin paper	0.01	0.01	0.01	0.03
2.	Disinfectants	0.05	0.05	0.05	0.15
	Sub Total B2:	**0.06**	**0.06**	**0.06**	**0.18**

Other Items:

S.No.	**Item**	**1st Year**	**2nd Year**	**3rd Year**	**Total**
B3	Travel	-	-	-	-
B4	Contingency	0.05	0.05	0.05	0.15
	Total	**0.05**	**0.05**	**0.05**	**0.15**
Sub-total(B1+B2+B3+B4etc.)		**0.06**	**0.06**	**0.06**	**0.18**
Grand total (A+ B1+B2+B3+B4)		**0.11**	**0.11**	**0.11**	**0.33**

PART-V: EXISTING FACILITIES

19.Available equipment and accessories to be utilized for the project :

Sl. No.	**Name of the Equipment/ Accessory**	**Make**	**Model**	**Funding Agency**	**Year of Procure ment**
1.	Gel documentation system	UVP		CSB	1997
2.	Refrigerated water batch circulator	Jeica Tech	RW 1025 G	CSB	2004
3.	Ice flakers	Simag	SPR 80	CSIR	2005
4.	P^H meter	Thermo	420 A	CSB	2005
5.	Deep freeze(-80 ^{0}C)	Haraeus	HFU 486	CSIR	2006
6.	Vertical gel electrophoresis system	Omega, Japan	-	CSB	2002
7.	UV spectrophotometer	Shimadzu	-	CSB	1994
8.	Micro-pipettes	Eppendorf, Tarson	-	CSB	2004
9.	Micro centrifuge	Hermile	-	CSB	2002

10.	Refrigerated Centrifuge (high speed)	Sorvall	-	CSB	1992
11.	Refrigerators	LG	GLD 32	CSB	2005
12.	Computer	HCL	-	CSB	2004
13.	Micro-centrifuge	Hermile	-	CSB	2002
14.	Electronic top loading balance	Sartorious	GE 812	CSIR	2005
15.	Deep freeze (-35ºC)	Remi	RQFV-265 (D)	CSB	2006
16	Environmental chamber	S.D.Instruments	HTC-3005	CSB	2014

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PART VI: BIODATA OF PROJECT COORDINATOR /PRINCIPAL INVESTIGATOR / CO-INVESTIGATOR(S)

1. Full Name (in Block letters) : DR.N.SURESH KUMAR
2. Designation : Scientist-D
3. Department/ Institute/ University: Silkworm Breeding and Genetics, CSRTI, Berhampore
4. Address for Communication : Silkworm Breeding and Genetics, CSRTI, Berhampore-742101

5. Date of birth : 01-06-1956
6. Sex : Male
7. Education (Post Graduation onwards & Professional Career):

Name of the University	Degree Passed	Year of Passing	Subjects taken with Specialization	Class/ Dvn.
University of Kerala, Trivandrum	BSc.	1978	Zoology (Main) Botany,Chemistry (Subsidiaries)	I Class
University of Kerala, Trivandrum	MSc.	1980	Zoology	II Class
University of Madras, Chennai	Ph.D	1986	Zoology (Entomology) Title: " Bio-ecological studies on some insects predatory on thrips (Thysanoptera : Insecta)" Guide : Prof. T.N.Ananthakrishnan, Formerly Director, Zoological Survey of India	

7. Awards:
[Not required for in-house personnel]

Year	Award	Agency	Purpose	Nature

8. Positions Held / Research Experience in various institutions:
[Not required for in-house personnel]

Employer	Designation of the post held	Date of Joining	Date of leaving

9. Memberships/Fellowships: [Not required for in-house personnel]

10. Patents: [Not required for in-house personnel]

11. Publications (Numbers only):
    Books: 05
    Research Papers, Reports: 100
    General articles: 20

List of important publications whose contents can be used in the proposed area of work:

10 important publications

1. **Suresh Kumar, N**, Kishor Kumar, C.M, Basavaraja,H.K, Mal Reddy, N, Ramesh Babu,M and Datta, R.K (1999) Comparative performance of robust and productive bivoltine hybrids of *Bombyx mori* L under high temperature conditions. *Sericologia.* **39**(4) : 567-571

2. **Suresh Kumar, N**; Yamamoto, T; Basavaraja, H.K and Datta, R.K. (2001)Studies on the effect of high temperature on F1 hybrids between polyvoltine and bivoltine silkworm races of *Bombyx mori* L. *Int. J. Indust.Entomol*. **2 (2)** : 123-127

3. **Suresh Kumar, N,** Basavaraja, H.K, Kalpana, G.V., Mal Reddy, N, Jayaswal, K.P., Thippeswamy, T., and Datta, R.K. (2002) Cocoon filament size deviation in bivoltine silkworm, *Bombyx mori* L. *Indian J.Seric.*, **41(1**) : 42-48.

4. **Suresh Kumar, N** , Basavaraja, H.K, Kishor Kumar, C.M., Mal Reddy, N., and Datta, R.K. (2002) On the breeding of " CSR18 x CSR19"- A robust bivoltine hybrid of silkworm, *Bombyx mori* L. for the tropics . *Int.J.Indust.Entomol*. **5(2)** : 155-162.

5. **Suresh Kumar, N**.; Basavaraja, H.K.**;** Mal Reddy, N. and Dandin, S.B. (2003) Effect of high temperature and high humidity on the quantitative traits of parents, foundation crosses, single and double hybrids of bivoltine silkworm, *Bombyx mori* L. *Int. J.Indust. Entomol*., **6**(2) : 197-202.

6. **Suresh Kumar, N**., Basavaraja, H.K., Kalpana, G.V. Mal Reddy, N., and Dandin, S.B. (2003) Effect of high temperature and high humidity on the cocoon shape and size of parents, foundation crosses, single and double hybrids of bivoltine silkworm, *Bombyx mori* L. *Indian J. Seric.***42** (1) : 35-40.

7. **Suresh Kumar, N**., Basavaraja, H.K., Kalpana, G.V. Mal Reddy, N.,, Kariappa, B.K and Dandin, S.B. (2003). Evaluation of bivoltine breeds and hybrids as male component with Pure Mysore under different temperature and humidity conditions. *Indian J. Seric.***42** (1) : 41-45.

8. **Suresh Kumar, N**., Basavaraja, H.K., Kalpana, G.V. Mal Reddy, N.,, Kariappa, B.K and Dandin, S.B. (2003). Evaluation of bivoltine breeds and hybrids as male component with Pure Mysore under different temperature and humidity conditions. *Indian J. Seric.***42 (1)** : 41-45.

9. **Suresh Kumar, N**., Basavaraja, H.K. and S.B. Dandin (2004). Breeding of robust silkworm, *Bombyx mori* L. for temperature tolerance – A review. *Indian J. Seric*., **43(2)** : 111-124.

10. **Suresh Kumar, N.;** Basavaraja,H.K.; Joge, P.G.; Mal Reddy, N.;. Kalpana, G.V. and Dandin, S.B.(2006) Development of a new robust bivoltine hybrid (CSR46 x CSR47) of *Bombyx mori* L. for the tropics . *Indian J.Seric*.,**45(1)** : 21-29.

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

Sl. No.	Title of the project	Funding agency	Duration From To	No. of Scientists/ Associates working under the project	Total approved cost of the project
1	Development of bivoltine silkworm breeds/hybrids suitable to adverse climatic conditions of Eastern India	Central Silk Board	2010 to 2015	Nine	

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

1. The hybrids viz., **CSR2 x CSR4 and CSR2 x CSR5 (**shell ratio >23.0% and raw silk % **-**19-20 ***and 2A to 3A grade***) were authorized (1997) and are being popularised on a large scale at farmers level during favourable months (Sept-Feb).

2. Three productive hybrids **CSR12 x CSR6, CSR3 x CSR6** and **CSR16 x CSR17** (raw silk % 18- 20 and 2A to 3A grade) were authorized during 1999 for commercial exploitation during favourable months. .

3. Two double hybrids, **(CSR6 x CSR26) X (CSR2 x CSR27)** for easy rearing of foundation crosses at P1 level with high egg recovery (10 –15 % more) are developed. The hybrid, **(CSR6 x CSR26) X (CSR2 x CSR27)** was submitted for Race Authorization test.

4. One robust hybrid **CSR18 x CSR19** (survival > 80 % at 36 ± 1 °C) has been authorized during 1999 for rearing throughout the year and is being popularised on a large scale at farmers level.

5. One more robust hybrid, **CSR46 x CSR47** (survival > 80 % at 36 ± 1 °C) with better productivity traits than CSR18 x CSR19 has been developed.

6. The productive bivoltine hybrid, **CSR48 x CSR5** with raw silk (20.6%), longer filament length (> 1300 m), thin filament size (2.45 d), relatively tolerant to high temperature (77% survival at 36 ±1°C and 85±5% RH) and low boil-off loss (23.8%) has been

7. The breed **CSR8(SL)** with sex-limited for cocoon colour for easy sex separation based on cocoon colour at grainages (yellow cocoons females and white cocoons males) has been developed.

8. To avoid the misuse of CSR2 normal breed as male component with PM, the new breed **"CSR2 (SL)"** with sex-limited for cocoon colour for easy sex separation based on cocoon colour at grainages (yellow cocoons females and white cocoons males) has been developed.

9. One more robust hybrid**, CSR50 x CSR51** tolerant to high temperature and tolerant to silkworm diseases have been developed

PART VI: BIODATA OF PROJECT COORDINATOR / PRINCIPAL INVESTIGATOR/ CO-INVESTIGATOR(S)

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.	-

7. Awards: [Not required for in-house personnel] Not applicable

Year	Award	Agency	Purpose	Nature

8. Position held/research experience in various Institutions: [Not required for in-house personnel]

Employer	Designation of the post held	Date of joining	Date of leaving

9.	Memberships/Fellowships: [Not required for in-house personnel]	
10.	Patents: [Not required for in-house personnel]	
11.	Publications (numbers only)	19 Papers and 35 Technical Reports
	List of important publications whose contents can be used in the proposed area of work	

LIST OF IMPORTANT PUBLICATIONS RELATED TO SERICULTURE:

- 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., 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 (edt). ***10: 717-724.***

- **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.**

- 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., **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.**

- **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**

- 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 pursued / carried out by Investigator:

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.*	CSIR, Delhi	April, 2002 to March,2005

3	*mori*. L.	CSB	Sept.,2004-Dec06
4	On farm trial of congenic silkworm hybrids for commercial exploitation	CSB	Dec' 05-Nov' 2007
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.	CSB	2005-2008
6	Mulberry Silkworm race Authorization Programme (MSRAP) – Phase- vii	CSB	Dec. 2007-Mar. 2010
7	Multi location Trial of New Silkworm Breads/Hybrids at Farm Level	CSB	Ph.I:07-10 PhII:10-13
8	Institute Village Linkage Programme (IVLP)	CSB	Aug.2010--Jul2012
9	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	2010-12
10	Validation trial of the Ready Reckoner of sulphur fertilizer application for obtaing targeted yields of mulberry	CSB	2012-14
11	Validation trial of technology for Prevention of Gattine disease in hot spot areas in silkworm *B. mori* L.	CSB	Jul, 2013 - Jun2016
12	Development of Multivoltine silkworm breeds with high shell percentage and neatness of silk filament	CSB	Jun,2012 - Dec2014
13	Post Authorization Trial of Silkworm hybrids in Eastern and North-Eastern India	CSB	Jul,2013-Jun,2016
14	Survey and surveillance of silkworm diseases in traditional districts of West Bengal	CSB	Apr,2013-Mar,16
15	Silkworm disease monitoring of seed and commercial crop rearing of West Bengal	CSB	Continuous.
16	Maintenance of Multivoltine and Bivoltine Germplasm.	CSB	Jun,2014-Dec2016
17	Pre-Authorisation Trial of Silkworm hybrids in Eastern and North-Eastern India	CSB	Sept.2012 – Aug.2016
18	Development of silkworm *Bombyx mori* L. Breeds from a gene pool with higher genetic plasticity	CSB	Jun.2014 - May2016

	Development of multivoltine congenic / NIL breed of silkworm *Bombyx mori* L. Through introgression of Id gene and its use		Jun.2014 - May2016

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-naphthylacetate 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)

LIST OF PUBLICATIONS

1. **Verma, A. K,** Ghatak, S. S. and Mukhopadhyay, S. (1990) Effect of temperature on the development of whitefly *B. tabaci* G. (Homoptera : Aleyrodidae) in the plains of West Bengal. **J. Agric. Sci. 60(5): 332-338.**

2. **Verma, A. K,** Basu, D., Nath, P. S., Das, S., and Mukhopadhyay, S. (1991) Some ecological consideration of whitefly and whitefly transmitted virus diseases of vegetables in West Bengal. **Ind. J. Virology.**

3. **Verma, A. K,** Basu, D., Nath, P. S., Das, S., Ghatak, S. S. and Mukhopadhyay, S. (1989) Relationship between the population of whitefly *B. tabaci* G. (Homoptera : Aleyrodidae) and incidence of tomato leaf-curl virus disease. **Ind. J. mycol. Res., 27(1), 49-52.**

4. **Verma, A. K,** and Ghatak, S. S. (1989) Influence of virus infected host plants on the biology of whitefly *B. tabaci* G. (Homoptera : Aleyrodidae) . **Environment & Ecology, 7(3): 736-639.**

5. **Verma, A. K,** and Singh, S. S. (1995) West Garo Hills a promising area for muga culture. **Indian Silk, Dec., 1995: 32-33.**

6. 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.**

7. 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.**

8. 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.**

9. **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.**

10. 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**

11. **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**

12. 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.**

13. 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, 17^{th}-18^{th}, January, **91-96.**

14. **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. 17^{th} -18^{th} January. Central Sericultural Research & Training Institute. Berhampore-742101, West Bengal: **97-100.**

15. 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: **"20^{th} Cong. of Intern. Seri. Commission", Bangalore. Vol .I, 268- 272.**

16. **Verma,A.K;** Mitra, P., Saha ,A.K., ghatak, S. S. and Bajpai, A. K.(2011) Effect of trap crop on the population of whitefly *Bemisia tabaci* (Genn.) and the diseases transmitted by it. **Bull. Ind. Acad. Seri., Vol. 15(2), 99-106.**

17. **Verma,A.K;** Bindroo, B.B and Saha ,A.K (2013) Bangalgram- A prospective village for bivoltine sericulture. **Indian Silk, April, 2013, Vol. 3(51old) No. 12, 6-8.**

18. 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.**

19. 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 October 2015).

20. **Verma,A.K,** Saha ,A.K., Nirmal Kumar, S., Chatterjee, G.K. and Suresh Kumar, N. (2014) Umar Faruk-A mirror of continuous struggle and spectacular success. **Indian Silk** (communicated).

21. Chatterjee, G.K., **Verma,A.K,** Saha ,A.K., Nirmal Kumar, S and Suresh Kumar, N. (2014). Esterase α, β - the biochemical markers for quantitative and qualitative traits of Silkworm, *Bombyx mori* L**.** ***Bangladesh Jr. Seric*.** (Communicated).

ABSTRACTS

1. 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.

2. 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) Silkworm breeding for tropical conditions of Eastern India .12 th All India Congress of Cytology and Genetics. Institute of Science, Mumbai, S-4.

3. 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.

4. 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**.**

5. **Verma,A.K;** Bindroo, B.B Santhakumar, M. V. and Saha ,A.K (2013) Inner flux and distribution of whitefly, *B. tabaci* G. (Homoptera : Aleyrodidae). In: *Proceeding of the 100th Session of the Indian Science Congress. University of Calcutta, Kolkata: EP P-03, p.-385.*

6. Dutta, S. K., Santhakumar, M. V., Verma,A.K, Ghosh, M. K., Bindroo, B.B. and Saha, A.K. (2012) Study on Lacipdiplodia theobromae, a root rot fungi of mulberry (Morus alba L.) in Malda district and its prophylactic measure. In: National Seminar on Recent Advances In Life Science Application (Dec.08rd - 09th). PG Department of Zoology. Acharya B. N. Seal College, Cooch Behar, Abstract No.-22, p.-21. Kalyani-741235. West Bengal.

7.

PGDS Dissertation-4

1. PGDS Dissertation of Sri M. Mohan Babu entitled "**A case study on Commercial Crop rearing of silkworm breeds (Multi x Multi & Multi x Bi) in commercial crop of Murshidabad district" (2004)**.

2. PGDS Dissertation of Md. Nasim Reja entitled "**A Case Study on Performance of Bhaduri Crop in West Bengal and suggestions for improvement**" **(2006)**.

3. PGDS Dissertation of Sri A. R. Sharma entitled " **A case study on management of silkworm seed at farmers level in respect of carrying of layings, incubation and black boxing** " (2013).

4. Smt. Gotimayum Pratima Devi entitled " **Testing of New Multi x Multi (V3 x V3) Hybrids of silkworm- *Bombyx mori* L." (2014).**

PART-VII: 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 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 of Biotechnology before implementing the project.
g. It is agreed by us that any research outcome or intellectual property right(s)on the intervention (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 investigators through out the duration of the project .
j. The institute assumes to undertake the financial and other management responsibilities of the project.

1. Signature of Executive Authority of Institute with Seal
Date:

2. Signature of Principal Investigator

3.Signature of Co-Investigator