

# CONSERVATION STRATEGIES AND SUSTAINABLE USE OF SERI- BIODIVERSITY WITH SPECIAL EMPHASIS ON MORI-SILKWORM (*Bombyx mori* L)

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## ABSTRACT

The main aim of seri-biodiversity conservation is to protect the available different ecotypes from extinction and use these in future breeding programmes. The extinction of traditional Indian silkworm races (Kashmir race, Chotopolu etc.) has only been due to lack of conservation/management system. The scientifically maintained, characterized and evaluated germplasm is therefore, of utmost importance to have specific breeding plans for upgrading the productivity and production stability. This could be met by laying a strategy involving both conventional and genetic engineering/molecular biology techniques to alter the genetic make-up of breeds more quickly, efficiently and precisely to evolve productive and stress tolerant breeds. This will also enable cost effective and efficient gene bank management and reduce the number of duplicate accessions to a manageable size.

Thus, germplasm collection needs to be identified, characterized and conserved, giving full attention to the conservation of local races also. Besides, characterization of silkworm germplasm is required to be documented properly so that information could be retrieved at any time for further use.

**Key words:** Biodiversity, Conservation, Silkworm (*Bombyx mori* L)

## INTRODUCTION

Conservation of biodiversity commonly called as “library of life” is an issue of global importance being addressed all over the world. The issue gains importance for our country which ranks among the 12 top biodiversity rich countries of the world and is probably the

richest centre of seri-biodiversity in the world hosting all the 5 commercially exploited types of natural silks along with their wild relative (Table1). Of the 5 silks, temperate tasar, tropical tasar and muga are still in wild form and are reared outdoor. The remaining, mulberry and eri, are reared indoor with systematic cultivation of food plants.

Human society depends on biodiversity for its diverse values, viz., food, fiber, fuel, shelter, medicines and several other day to day necessities without which sustenance of mankind on this earth is impossible. It is, therefore, pivotal to enhance understanding, augmentation, conservation and sustainable use of biodiversity with appropriate human interventions.

Prior to the emergence of GATT, developed countries used to exploit biodiversity of developing countries. It has now become important under GATT to prevent exploitation of developing countries which are rich in biodiversity and traditional knowledge (TK) by the techno-economically rich and developed countries. It has also become important under GATT to characterize the diverse germplasm using the techniques that give highly reproducible results. Because of this fact, the historic Rio Earth Summit of 1992 rightly recognizes the sovereign rights of the nations (States) over their biodiversity. This was adopted by 174 countries and India, which is one of the signatories to the agreement, adopted this resolution in 1992. The CBD also reaffirms that:-

- the States are responsible for conserving their biodiversity
- the developed countries can use the biodiversity only after prior agreed consent to prevent any eventual misuse or patenting by others.
- the vital role of communities especially women is recognized
- the biodiversity is used in a sustainable way without causing damage to the ecosystem.
- the desirability of sharing the benefits arising from the use of TK, skill and

innovations equitably along with the biodiversity.

- the importance of and need to promote regional and global co-operation for conservation and
- the requirement of substantial investments to conserve biodiversity.

This indicates the importance of biodiversity for sustainable living of mankind on the earth.

**Table 1: Types of silkworms present in India**

Scientific name	Common name	Origin	Food plant
<i>B. mori</i>	Mulberry silkworm	China	<i>M.alba, M. indica, M. multicaulis, M.bombycis</i>
<i>A.mylitta</i>	Tropical tasar silkworm	India	<i>Shorea robusta, Terminalia tomentosa, T.arjuna</i>
<i>A.proylei</i>	Oak/temperate tasar silkworm	India	<i>Quercus incana, Q.serrata, Q.himalayana</i>
<i>A.assama</i>	Muga silkworm	India	<i>Litsaea polyantha, Machilus bombycine</i>
<i>P.ricini</i>	Eri silkworm	India	<i>Ricinus communis, Maninot utilisma, Evodea fragrance</i>

**IMPORTANCE OF SERI-BIODIVERSITY CONSERVATION**

Sericulture is one of the major commercial activities of India earning annual foreign exchange to the tune of Rs. 2294 crores (2002-03). The value of silk export earnings has been anticipated by Govt. of India (Ministry of Textiles) to be around 2700 crores during the coming years. However, the present production of raw silk falls very much short of domestic demand. To meet this demand as well as to enhance the quantity and quality of

silk export, silkworm germplasm has to play a pivotal role.

The maintenance and sustainable use of all beneficial plants, animals, organisms, involves the development of a reservoir of gene assemblies or “Gene Library”. The gene reservoir consists of geographically isolated races, genetic stocks, breeds evolved from exotic/indigenous crosses or hybrids besides the breeds/races collected from different centers/institutions.

Sericulture is one of the important and viable economic activities in developing countries like India. In order to sustain and meet the future demands of this agro-based activity, it should efficiently be backed up by the: –

- Directional improvement and
- Conservation of silkworms and its host plant/s

Several countries and institutions are actively involved in evolving collecting/maintaining the biodiversity. So far as the seriodiversity is concerned, it is at presently being maintained by some important institutions of the world.

- The Laboratory of silkworm Stock Culture of Kabuchizawa in Yamanashi Prefecture, Japan maintains about 500 silkworm breeds.
- The MRIS Institute of Russia has a stock culture of 197 breeds.
- Sericulture Research Institute of China maintains about 800 silkworm breeds.
- In India more than 460 bivoltine and 150 multivoltine breeds are maintained at different breeding centers/research institutions.

#### **NATIONAL GERMPLASM ACTIVE SITE**

Central Sericulture Germplasm Resources Centre, Hosur is the biggest reservoir of seriodiversity in India and has been recognized as 'National Active Germplasm Site' (NAGS) for mulberry and silkworm by National Bureau of Plant Genetic Resources (NBPGR), National Bureau of Animal Genetic Resources (NBAGR) and Indian Council of Agricultural Research (ICAR). The centre, which maintains 401 breeds of mori- silkworm that include 71 multivoltine and 330 bivoltine breeds besides 20 mutants, has also analyzed morpho-physio-biochemical parameters of some mulberry and silkworm accessions. The centre has also documented silkworm genetic resources, evaluation data base management and list of descriptors in "The Catalogues of Silkworm

(*Bombyx mori* L.) Germplasm. Even S. K. University of Agricultural Sciences and Technology of K (SKUAST-K) is maintaining about 151 genotypes of bivoltine silkworms of diverse geographical origin. This includes the breeds numbering 25 developed at its Sericulture Division during 1982 – 1999. Out of these 2 varieties, viz., SKAUR-1, SKAUR-6 and their hybrid SKAU-HR-1 have been released during 1995 by the Ministry of Textiles, Govt. of India, for commercial exploitation in J&K State (Kamili, 1996) and others like SKUAST-27, SKUAST-28, SKUAST-29, SKUAST-30, SKUAST-31, SKUAST-32, SKUAST-33 and SKUAST-34 are showing superiority in one or other characters (Kamili *et al.*, 2000) and are at pre – release stage. In near future, their number is expected to increase with diverse qualitative and quantitative features. Dr. Tazima, a leading Japanese sericulturist during his visit to India in 1957 has expressed that Kashmir could be converted into silkworm gene bank for sustaining the sericulture of the whole world in view of its salubrious climatic conditions (Kamili and Masoodi, 2000)

The importance of conservation of breeds is compulsory not only for initiating new breeding programmes but also for conserving the old races which have gone out of culture. Special emphasis needs to be given on saving the genetically important strains from extinction and exploring new strains for the development and progress of country's silk industry. Had there been the conservation programme of seriodiversity earlier, we would not have lost some precious seriodiversity like Barapalu, Chatapalu and Kashmir Race. Kashmir Race had been the productive indigenous univoltine breed of Kashmir about 130 years back but has now become extinct due to out break of deadly Pebrine disease. Such has been the production and productivity of this breed that in 1869 British managed to export from Kashmir (India) thousands of ounces of disease free

seed of this Race to Europe in order to revive sericulture there.

With the development and release of new breeds and hybrids, the older races are even today facing the danger of being lost due to careless handling, difficulties in managing the large stocks and in the absence of efficient renewal programmes vis-a-vis the excessive use of pesticides polluting the environment and disturbing the ecosystem, emergence of diseases and pests and use of single method of breeding that has put the seri-biodiversity to a greater threat. If sufficient care and management is not ensured now we may lose much more important genetic material.

Traditionally genetic variation was assessed merely by the analysis of morphological traits. It has been reported that 330 bivoltine and 63 multivoltine silkworm breeds have been morphologically characterized for their egg, larval, cocoon, pupal and adult stages (Thangavelu *et al.*, 2000). These non-numeric parameters also play a vital role in the identification, accessioning and maintenance of the germplasm (Kumaresan *et al.*, 2004). Characterization on the basis of morphological traits alone may not lead to sound judgment and discrimination of duplicate accessions. As such conservation of breeds is required to be based on more advanced reliable, quicker and unambiguous methods with high precision like DNA analysis, genomic analysis, biochemical markers, etc. In the present era of frontier sciences, there is need for identification and characterization of molecular/genetic attributes of different silkworm breeds, besides the use of conventional methods of breeding so as to have quicker and targeted improvement of silkworm breeds for their utilization in enhancing the production and productivity of cocoons with the ultimate objective of economic sustainability of farmers (Kamili *et al.*, 2003). The plan for conservation and improvement of the seri-biodiversity therefore, should be based on the following biotechnological interventions.

### A. Biochemical markers for the improvement of traits

Prospects of using biochemical markers (isozyme/DNA) for the improvement of desirable traits in plants and animals have received attention of breeders. Identification of suitable biochemical markers is essential for Marker Assisted Selection (MAS) being used in various fields of breeding with a view to make sericulture economically viable. Gene linked traits like growth rate, cocoon yield, fibre quality and resistance to various disease and pests could be tagged with molecular markers for rapid development of improved strains (Goldsmith, 1995). The genetic variation estimated through the isozyme profiles based on differential allozymes expression in various tissues of some silkworm accessions have shown 4 allozymes (G6DP, a', b' esterase and acid phosphatase) that could show genetic variation through allelic frequency of alleles (Asok Kumar *et al.*, 2004). The molecular markers, both DNA sequences and Isozymes, are useful because these do not have much negative effect on phenotype.

#### (i) *Amylase as a marker*

In silkworm, out of a number of biochemical parameters, digestive amylase has been found to play significant role on the expression of various attributes. Chatterjee *et al.* (1992) have found positive correlation of digestive amylase with better digestibility and survival. Thus, better digestibility indicates prospect of using amylase as a marker in silkworm breeding.

#### (ii) *Transfer of amylase gene*

It has been reported by many molecular biologists that polyvoltine races contain dominant amylase gene ( $AC^+$ ) which is responsible for inducing hardiness in multivoltine breeds. Conversely,  $AC^-$  gene is recessive in temperate breeds due to which these breeds do not tolerate much environmental fluctuations especially high temperature and humidity. Breeders, however,

can transfer dominant ( $AC^+$ ) gene of polyvoltine breeds into temperate breeds through a suitable vector for developing hardy univoltine/bivoltine races (Kamili and Masoodi, 2000).

### (iii) *Proteinase as a marker*

Studies have also reported that gene controlling proteinase in digestive juice is positively correlated with dietary efficiency of silkworm. The dietary efficiency is reported better in bivoltine/univoltine races. Thus proteinase of efficient breeds could be used as a marker in improving the yield.

## B. Genes and disease resistance

It is imperative for all the breeders to characterize and evolve disease resistant breeds of silkworm to overcome the losses due to various diseases. It has been reported that NPV in *B. mori* (Bm NPV) is controlled by one dominant and CVP (B cm CPV) by another gene (Watanabe, 1967). The genes responsible for offering such resistance could be isolated through DNA probe and c DNA could be transferred to the host cell through vector such as NPV by Recombinant DNA Technology. The host cell incorporating foreign DNA in its chromosome could impart disease resistance. Thus, such traits could be identified and characterized for their utilization in the evolution of disease resistant breeds.

## C. Identification of anti-microbial proteins

Insects are amazingly resistant to microbial (bacteria, fungal, etc.) infections. To combat pathogens, insects rely on cellular and humoral mechanisms. Upon detection of a microbe, a complex genetic cascade is activated which ultimately results in synthesis of battery of antibacterial peptides and their release into haemolymph. The majority of antimicrobial peptides act through disintegrating the bacterial membrane or interfering with the membrane assembly. These antimicrobial proteins have wide application in therapeutics and could be used against microbial infections besides being used as food and feed preservatives.

Attempts have been made at various institutes (both at national and international levels) to isolate antimicrobial proteins from various silkworm varieties for various uses. Recently antibacterial proteins have been isolated from *B. mori* larvae in response to gram negative strain of bacteria (*E. coli*) in biotechnology laboratory of SKUAST-K. 10KDa and 70KDa antibacterial proteins have been successfully isolated (Fig. 1& 2) from SKUAST-6 and SKUAST-1 strains of silkworms (Kamili *et al.*, 2004). After analyzing these proteins by amino acid sequencing and cloning these could be used for various applications.

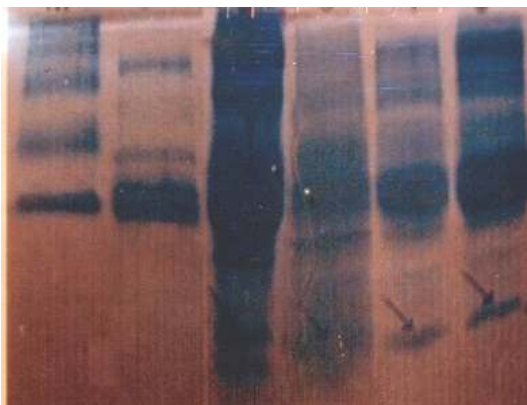


Fig. 1. Lane 3,4,5 showing 10 KDa anti-*E.coli* protein secreted by SKUAST-6 strain of silkworm

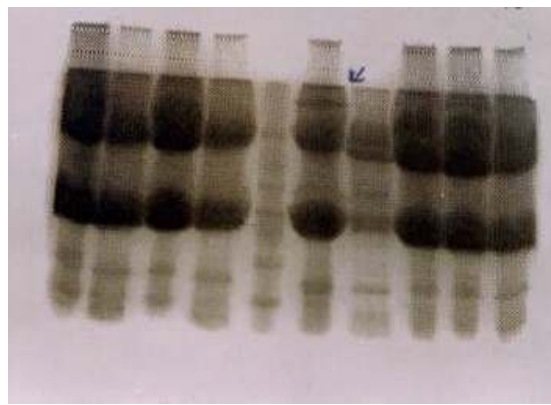


Fig. 2. Lane 5 showing 70 KDa anti-*E. coli* protein band secreted by SKUAST-1 strain of silkworms

#### D. Characterization of thermo-tolerant breeds

Spring is the conducive season in our state for rearing monovoltine / bivoltine breeds of silkworms. Summer season, when temperature and relative humidity remain high, is hardly suitable for conducting rearing of these breeds. Keeping this in view, characterization of thermo-tolerant breeds is imperative to utilize these in the breeding plan to evolve productive breeds tolerant to high temperature and humidity. Studies carried out by several Japanese scientists have indicated that certain enzymes in the silkworms are responsible for high temperature tolerance (Sarkar, 1998).

##### (i) Heat Shock Proteins (hsp)

Various Heat Shock Proteins (hsp) also contribute to thermo tolerance in silkworm. It has also been reported that thermo tolerance varies with the breeds and is positively correlated with the activity of heat stable esterases (Hs EST) analyzed in the mid gut. Tolerance to high temperature is a heritable character which envisages evolution of suitable silkworm breeds tolerant to high temperature. Besides, there is a heritable linkage between high temperature tolerance and disease resistance. Thus, the breeds with these characteristics could be identified and characterized in the germplasm as a resource material for developing thermo tolerant breeds/hybrids.

#### E. Quantitative Trait Loci (QTL) based molecular characterization

Not much is known about the genetic factors that influence the traits contributory to the yield. It is, however, very clearly known that:

- The quantitative traits are highly divergent in poly and bivoltine breeds and
- Economic traits are poly genic, i.e., these are controlled by many genes.

Very recently, mapping and analysis of QTL has been possible with the aid of linked genetic markers mostly molecular (Isozyme/DNA). While the extensive linkage analyses in silkworm during the last several decades have resulted in a linkage map of 217 loci (Doira *et al.*, 1992), these have mostly remained objects of academic interest or used only in evolving sex-limited breeds. However, DNA markers (molecular mapping) have very recently been used for mapping the QTLs in various plants and animals. In silkworm, the development of molecular map has just begun and the completion of RFLP/RAPD (Restriction Fragment length polymorphism/Random Amplified Polymorphic DNA) maps may require some time more. Once the genetic markers closely linked to QTLs controlling various economic traits are developed, these could be used as molecular tags in silkworm breeding for the improvement of target traits

#### F. Genome analysis using molecular markers

Genome analysis of *B. mori* using molecular markers has also been initiated recently. Application of PCR based RAPD and DNA finger printing to study the DNA profiling of silkworm genotypes with mini satellite probes has also been started at Seri-Biotech Laboratory, Bangalore. It has been reported that each genotype revealed distinct and unique DNA profile specific to diapausing and non-diapausing strains. These studies indicated their

potential use not only in understanding genetic relationship but also as powerful tool to generate markers that are linked to traits of interest in silkworm. Thus use of DNA markers like RFLP for studying the DNA profile in the germplasm for characterization at molecular level could prove highly beneficial. Seri-Biotech Laboratory, Bangalore has succeeded in finding primers associated with characterizing low and high yielding races.

#### **G. Other characters of interest in silkworms**

Besides the above explained genetic resources, the following breeds with special characters are also required to be identified and characterized for their utilization in target based breeding programmes to meet the industrial requirements.

- Breeds with unique morphological features.
- Breeds with higher neatness, raw silk% and cocoon yield.
- Breeds with high egg index and egg production efficiency.
- Breeds with less size filament deviation, lousiness and low boil off loss.
- Breeds with thin/moderate denier (2-3) and longer bave length (above 1200m)
- Viable/ potent sex-limited breeds
- Breeds showing tendency of being polyphagus
- Breeds having different larval and cocoon color as well as markings.
- Breeds showing preference to artificial diet.

Having large collection of germplasm by itself has no significance unless the collection is carefully studied for its genetic representatives so that duplicity is avoided and useful traits assessed for economic improvement. This requires a multidisciplinary evaluation programme so as to harvest maximum benefits from valuable assemblage of genotypes.

#### **H. Sustainable use of seri biodiversity**

Conservation without effective utilization is useless. There is urgent need for increased utilization of seri biodiversity both for commercial use and experimental use to increase the silk production and to evolve better yielding breeds.

## I. Documentation and publication

In order to create better awareness regarding activities of sericobiodiversity conservation and improvement at different centers, it is necessary to bring out a Reporter twice a year for wide circulation which will furnish details on the material characterized, introduced, evolved as well as modes and means of utilization which will help breeders and other users to be assured that the varieties used are genuine. Central Sericultural Germplasm Research Centre has developed its own documentation system known as Silkworm Germplasm Information System (SGIS), where the primary data passport information and secondary data recorded through evaluation & characterization have been documented for further use at any given time. Similar exercise needs to be done at other research institutes.

## REFERENCES

- Ashok, Kumar K., Somasundaram, P., Kar, P. K. and Velu, D. 2004. *Biochemical characterization and evaluation of silkworm genetic resources*. pp. 104-105 Annual Report (2003-2004), Central Sericultural Germplasm Resources Centre, Central Silk Board, India,
- Chatterjee, S.N., Roa, P.P.M., Jayaswal, K.P. Singh., R. and Datta, R.K. 1992. Genetic variability in mulberry silkworm, *Bombyx mori* L breeds with low silk yield. *Ind. J. of Seric.* **32**(1):69-89.
- Doira, H., Fijii, H., Kawaguchi, Y. and Kihara, H., Banno Y. 1992. *Genetic stocks and mutations of Bombyx mori*. Institute of Genetic Resources, Kyushu University, Japan.
- Goldsmith, M.R. 1995. *Molecular model systems in the Lepidoptera*. pp. 21-76. In: *the 20<sup>th</sup> congress of the international sericulture commission Vol I*. M.R. Goldsmith and A.S. Wilkins, (ed), Cambridge Univ. Press, Cambridge,
- Kamili, Afifa S., 1996. *New bivoltine silkworm breeds and their hybrids (SKAU-HR-1)*. S.K. University of Agricultural Sciences and Technology (J&K).
- Kamli, Afifa S., Ahmad, Raies and Hussaini, Humaira. 2004. Induction of anti bacterial proteins in bivoltine silkworm (*Bombyx mori* L.) in response to gram – negative strain of bacteria. *Oriental sci.* **9**(2):73-76 .
- Kamili Afifa S., Malik, G.N., Trag, A.R., Kukiloo, F.A. and Sofi, A.M. 2000. *Development of new bivoltine silkworm (Bombyx mori L) genotypes with higher commercial characters. SKUAST-J Res.* **2**:1-95.
- Kamili, Afifa S. and Masoodi, M. Amin.2000. *Principle of Temperate Sericulture*. Kalyani Publishers, New Delhi.
- Kamili, Afifa S., Dalal, M.A. and Hussaini, H.2003. Need to bring transgenic crops to farmers. p 887-95. *Proceedings of National Symposium on Sustainability of Agricultural Production and Value Addition in the Context of WTO*. March, 20-21 *SKUAST- J. Res.*
- Kumaresan, P., Mohan, B., Koundinya, P.R., Sinha, R.K. and Thangavelu, K. 2004 Silkworm germplasm conservation – A perspective. *Indian Silk* **42**:8-11.
- Sarkar, D.D.1998. *The Silkworm Biology, Genetics and Breeding*. Vikas Publishing House Pvt Ltd, Jangpura, New Delhi.
- Thangavelu, K., Sinha, R.K., Mahadevamurthy, T.S., Radhahrisnan, S., Kumaresan, P., Mohan, B., Rayaradder, F.R. and Sekar, S. 2000



*Catalogue on silkworm (Bombyx mori L.) germplasm, Vol II. Central Sericultural Germplasm Resources Centre, Central Silk Board, Hosur, Tamil Nadu, India.*

Watanabe.1967.Development of resistance in silkworm, *Bombyx mori* L, to per oral infection of cytoplasmic polyhedrosis virus. *J. Invet. Path.* **9**: 474-479.