# Recent Breakthroughs in Sericultural Technology in India to Match the Requirement of Silk Industry in Tropics

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(Received 30 September 2000; Accepted 19 October 2000)

Strengthening of R & D components of sericulture in India since the sixties has led to a quantum jump in silk production and presently India is the second largest producer in the world. This achievement is primarily due to a number of breakthroughs in R & D to match the requirements of tropics, by way of introduction of improved mulberry varieties and silkworm breeds, better mulberry cultivation and rearing management practices suited to tropical conditions. Of late, new approaches in molecular biology and biotechnology have also been vigorously pursued to strengthen the current conventional strategies. The present paper attempts to provide an overview of the present status of silk production in both mulberry and non-mulberry sectors, breakthroughs achieved through new approaches of biotechnology and the future prospects for maximizing silk productivity in India.

**Key words**: Indian sericulture, Breakthroughs in R & D, Non-mulberry, Molecular biology, Biotechnology.

#### Introduction

India is the homeland of all the four types of natural silks, *i.e.*, mulberry, tasar, muga and eri. Though ground work for establishing sericulture industry in India was laid about 200 years back, building of a nationwide R & D infrastructure was started only during the sixties. Since then the research endeavours in sericulture have passed through an exciting phase of development. Initial drawbacks have been overcome through a number of scientific advances and technological breakthroughs. Dramatic

results have been achieved in boosting the silk production by ten folds since the sixties. This impressive progress is mainly due to various productive factors like increase in area, evolution of high yielding host plant varieties and silkworm breeds, better cultivation system and rearing management, control of pests and diseases *etc*. The breakthroughs in R & D have led to the development of technology packages suitable for tropics which has resulted in significant increase in the silk production in India. Bulk of the silk produced is of the mulberry variety, while less than 8% of the total production belongs to the non-mulberry type. Hence, the chief thrust of R & D has been focused towards mulberry sector than that of non-mulberry.

Pressures on natural resources like water and land have limited the horizontal expansion and hence the vertical growth is the only option to increase productivity. In this context, it is imperative to take note of the rapid developments in molecular biology and biotechnology which have offered solutions to the drawbacks in conventional approaches and have accelerated the productivity of crops. Hence, the situation demands the need to try these novel strategies to harness the benefits of their applications. The new tools of biotechnology have to be integrated with the traditional methods to augment the silk productivity in India to meet the requirement of both domestic as well as global demands.

### Present status of mulberry sericulture in India

#### Mulberry raw silk production

After independence, the mulberry area as well as the raw silk production in India progressed significantly and increased steadily which has shown a quantum jump in silk production by over ten folds since the 50s. Further, the silk productivity per hectare has also shown a significant leap with considerable reduction in renditta (Table 1).

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Table 1. Progress of mulberry raw silk production

Year	Area (ha)	Day Cill (M.T.)	Product	- Renditta	
	Alea (lia)	Raw Silk (M.T.) -	Raw silk (Kg)	Cocoon (Kg)	- Kendida
1950-51	56732	957	16.87	287.09	17.0
1960-61	82954	1185	14.29	240.23	16.8
1970-71	98248	2319	23.60	348.89	14.8
1980-81	170000	4593	27.02	342.40	12.7
1990-91	316610	11486	36.28	368.48	10.2
1999-2k	226349	14700	64.94	580.72	8.9

**Table 2.** Evolution of mulberry varieties

Variety	Year of Release	Yield/ha/Yr (MT)
Rainfed		
Local	Traditional	6-8
S13	1992	15-16
S34	1992	15-16
Irrigated		
Local	Traditional	15-20
Kanva 2	1970	30-35
S54	1986	40-45
S36	1991	35-40
<b>V</b> 1	1996	60-70

#### Mulberry production and cultivation

For a long time, 'Local' Mulberry variety was extensively in use which was producing only 6-8 MT under rainfed condition and 15-20 MT/ha/year under irrigated condition. Later, a number of mulberry varieties were evolved (Shastry, 1984) for both rainfed and irrigated areas and recently, a breakthrough in mulberry breeding has been achieved which has resulted in the evolution of V-1 variety which has the potential of yielding up to 70 MT(Jalaja *et al.*, 1996) and is qualitatively superior to the earlier varieties evolved (Table 2).

Improved agronomic practices for mulberry cultivation involving spacing, manuring and efficient irrigation management have been standardized and popularized which has improved the leaf yield and quality, facilitates mechanized cultivation and reduces the cost of production (Table 3).

### Silkworm breeds and rearing technology

Prior to the 70s, only multivoltine × multivoltine (Pure Mysore × C.nichi) races were reared which produced 15-20 Kg/100 dfls. With the introduction of bivoltines, there has been a shift in the farmers choice for cross breeds, *i.e.*, multi × bivoltines, which were easy to rear and less risky than bivoltine hybrids and an yield of 30 - 40 Kg/100 dfls could be realized. Even now, nearly 95% of the silk pro-

Table 3. Improved cultivation package

Spacing	$(90 + 150) \times 60 \text{ cm}$		
Manure	20 MT/ha/yr		
Fertilizer (kg/ha/yr)	350 N :140 P :140 K		
Irrigation	3.75 cm/irrigation once in 10 days		
Harvesting	Shoot harvest		
Leaf yield	70 MT/ha/yr (Variety V1)		

Table 4. Evolution of multivoltine silkworm hybrids

Year	Yield/ 2 boxes (Kg)	Cocoon shell Ratio (%)	Renditta
1950-70 1995	15-20 25-30	12-14 18-19	14-16 9-10
1996	25-30	18-19	8-9
1970-75 1975-80 1975-80 1996	25-30 35-40 35-40 45-50	15-16 17-18 17-18 18-19	9-10 9-10 9-10 8-9
	1950-70 1995 1996 1970-75 1975-80 1975-80	Year boxes (Kg)  1950-70 15-20 1995 25-30 1996 25-30  1970-75 25-30 1975-80 35-40 1975-80 35-40	Year boxes shell Ratio (%)  1950-70 15-20 12-14 1995 25-30 18-19 1996 25-30 18-19  1970-75 25-30 15-16 1975-80 35-40 17-18 1975-80 35-40 17-18

duced is of the cross breed type. Recently with the objective of improving the quality and productivity of cross breed silk, new polyvoltine breeds, *viz.*, RD1, BL23 and BL24 were evolved. The two hybrids, BL23×NB4D2 for rainfed areas and BL24×NB4D2 for irrigated areas have been identified, which have higher productivity than the earlier cross breeds (Table 4).

Inspite of the fact that bivoltines could be reared in the southern plateau, it did not make a significant dent because of cross breeds gaining popularity among the farmers. One of the main drawbacks of the bivoltine hybrids was the lack of mulberry varieties with quality

**Table 5.** Performance of productive bivoltine hybrids

Hybrid	Pupation Rate	Cocoon shell Ratio (%)	Renditta
CSR2×CSR4	92.1	23.4	5.2
CSR2×CSR5	89.8	23.6	5.2
CSR3×CSR6	90.5	24.4	5.1
CSR16×CSR17	93.4	23.0	5.6
CSR12×CSR6	94.7	24.1	5.0
CSR13×CSR5	89.7	24.2	5.1
CSR20×CSR29	92.7	23.1	5.2
KA×NB4D2(Control)	89.9	20.4	6.5

and quantity matching the requirement of bivoltines and also the low shell ratio of 18-19% realized at the commercial level. Improvement of qualitatively superior raw silk recovery necessitated the evolution of high silk yielding bivoltines.

With the collaboration of Japanese International Cooperation Agency (JICA), a breakthrough in breeding productive bivoltines has been achieved and hybrids have been identified having the potential to produce 2A to 4A grade silk (Table 5).

Among the seven hybrids, the first four have already been authorized for commercial production. During the II phase of JICA assisted programme, the hybrids CSR2×CSR4 and CSR2×CSR5 were tested under large scale with the farmers under tropical bivoltine technology package, which also included the improved mulberry varieties, cultivation system, new technology of rearing, disinfection and maintenance of hygiene *etc*. Over 80000 layings were reared under eight crops and an average yield of 65 Kg/100 dfls could be realized with a renditta of 5-5.5

and silk quality of 3A to 4A grade.

The productive breeds evolved were recommended for rearing during favourable months as the hot climate of the tropics are not conducive for rearing these elite breeds. Hence, breeds have been developed by screening and selection at high temperature (36°C) and humidity (85%) and the hybrid CSR18×CR19 has been authorized in 1998 for commercial exploitation throughout the year (Datta *et al.*, 1997).

Further, breeding for disease resistance has also been initiated. Silkworm stocks resistant to NPV and DNV 1 have been identified which will be used as donor parents for introgression of disease resistance into the genetic background of productive silkworm breeds.

Disinfection and hygiene during silkworm rearing plays a vital role in the prevention of diseases. Recently, chlorine dioxide mixed with lime, which is an effective and non-corrosive disinfectant, has been found to be the most suitable alternative to formalin and bleaching powder.

To prevent the spread of viral and fungal diseases during silkworm rearing, a unique bed disinfectant named "VIJETHA" was formulated which controls all the diseases, *viz.*, pebrine, flacherie, grasserie and muscardine in the rearing bed. Field trials have shown 10-15 Kg increase in the cocoon yield for two boxes of eggs.

### Present status of non-mulberry sericulture in India

India produces all the three types of non-mulberry silks, namely, tasar (tropical and temperate), eri and muga, which contributes less than 8% of the total silk produced presently by India. Out of the total silk production of over 1300 MT, bulk of the silk is of the eri variety followed by tasar and muga silks (Table 6).

**Table 6.** Current non-mulberry production and the species involved

Type of silk	Production (MT)	Silkworm	Host plant	Regions	
			Terminalia arjuna	Bihar, M.P.	
Tropical	290	Antheraea	T. tomentosa	Orissa, A.P.	
Tasar		Mylitta	Shorea robusta	Himalayan belt	
Temperate		A. roylei			
Tasar		A. proylei	Quercus serrata		
			Q. incana		
Eri	1000	Philosamia	Ricinus communis	North-East	
LII	1000	Ricini	Manihot utilissima	States	
Muga	80	A accama	Machilus bombycina	Assam	
iviuga	<b>6</b> U	A. assama	Litsea polyantha	Meghalaya	
Total	1370				

#### The tropical tasar silkworm

Antheraea mylitta exitsts in a wide range of geographical conditions and about 24 ecotypes have been identified, out of which only four of them, *i.e.*, Daba, Raily, Sarihan and Modal are commercially important for different ecological niches. A new technique of indoor rearing up to III instar under nylon net has been developed which prevents the loss of silkworms due to pests and predators in addition to biological control measures. For propagation of food plants, improved nursery techniques have been devised. For improving the reeling and quality of tasar silk yarn, cooking media have been formulated and improved reeling machines have been fabricated.

#### The oak tasar

A. proylei is a fertile interspecific hybrid of A. pernyi x A. roylei. The hybrid proved to excel both parents in all economic characters and thus gave rise to temperate tasar culture in India. A few pure lines are evolved through hybridization and backcrossing with A. pernyi and A. roylei. Indoor rearing of early instars have been standardized.

#### The muga silkworm

A. assama which produces the golden yellow silk, is reared only in Assam because of its characteristic ecological requirement of host plants which are endemic to Assam. Two morphotypes, S4 and S6 were found promising for large scale multiplication and raising of plantations. Improved propagation and nursery techniques have been developed. Reeling machines have been fabricated to replace the traditional reeling devices.

#### Eri silkworm

*P. ricini* is the only non-mulberry silkworm which is completely domesticated and feeds on castor plants. The wild relative *P. Cynthia* freely crosses with the domesticated variety but no heterosis could be found. Eri cocoons are not reelable but can only be spun by hand. New spinning machines have been fabricated.

Presently, various supportive measures to develop non-mulberry sericulture to help tribal economy and increase the silk production in India have been initiated (Subba Rao, 1998). Seed organization is being strengthened by establishing three tier multiplication of seed through basic seed multiplication centers. Grainage technology package for cocoon preservation, egg laying, egg incubation *etc.* have been standardised. To increase the food plant population, large areas of waste lands have been brought under cultivation of food plants of tasar, muga and eri silkworms. Raw material banks for cocoon marketing have been established to support remunerative returns to

cocoon growers.

# Need for alternative strategies to augment silk production

The findings of R & D strategies taken up so far have led to the development of appropriate sericulture technologies suited to the tropical climate. This has resulted in a phenomenal increase in productivity of silk over the last four decades. Presently, India is the second largest producer of silk in the world. However, bulk of the silk produced is of multivoltine × bivoltine variety which is of low category as far as international grading is concerned. During the last few years, nearly 5000 MT of bivoltine silk was imported to meet the requirement of local powerloom sector. Further, the silk productivity per hectare is about 65 Kg which is less than that of China (80 Kg) and far below than that of Japan (120 Kg). The conventional approaches alone undertaken so far are not sufficient to solve the problems in conventional practices and to increase productivity at a faster pace. Hence attempts have been made to seek solutions to the problems through biotechnological approach to supplement the present strategies.

# New strategies adopted in molecular biology and biotechnology

To improve the productivity of crop plants and livestock, rapid developments in the field of molecular biology and biotechnology have opened up new avenues. These have provided alternative strategies to overcome the limitations of conventional approaches. In Sericulture also, various novel strategies have been taken up during the last few years to boost the productivity.

#### Biofertilizers for reduction of chemical fertilizers

During 70s, package of practices for mulberry cultivation was recommended with an input of 20 MT of FYM/ha/ year plus 300: 120: 120 NPK under irrigated conditions and under rainfed conditions, 10 MT of FYM + 100: 50: 50 NPK was suggested. High cost of fertilizers and manures and their non-availability in time, led to the poor adoption and lower input at the farmers level. To overcome these drawbacks, alternative technologies have been developed and an integrated nutrient management approach is being recommended. Common micro-organisms like Azotobacter are capable of biological nitrogen fixation which contributes maximum N to the soil. Use of bio-fertilizers have been shown to save 50% of nitroge-

nous chemical fertilizer (Das *et al.*, 1996). On similar lines, use of Vesicular Arbuscular, Micorrhizal (VAM) fungi isolated from mulberry root zone has been found beneficial in cutting down phosphatic fertilizer usage by 75% (Katiyar *et al.*, 1995).

#### Biocontrol agents for mulberry and silkworm pests

The key pest of mulberry is the mealy bug, *Maconellicoccus hirsutus* causing apical shoot malformation known as "Tukra". The coccinellid predatory beetle *Cryptolaemus montrouzieri*, commonly known as the lady bird beetle has been identified to be an effective biocontrol agent against mealy bug. A single grub of the beetle consumes about 900 eggs or 200 nymphs or 30 adults of mealy bug during its life period. Further an IPM approach involving mechanical, chemical (0.2% DDVP in 0.5% soap solution) and biological control methods was found to significantly decrease the mulberry pest population.

Among the pests of the silkworm, predominant one is the uzi fly, *Exorista bombycis* which causes 10-20% damage depending on the season and region. *Nesolynx thymus*, a hymenopteran parasitoid of uzi fly has been identified as effective biocontrol agent (Kumar *et.al.*, 1996). *N. thymus* parasitises 1-8 day old pupae of uzi fly. A single adult female parasitises 4-5 host pupae during its life time and the number of adults emerging from the parasitised pupae varies between 30 and 60. An IPM package containing the use of nylon net, spray of uzicide, uzitrap and biological control measures have been found to minimize the uzi fly infestation.

Technology for mass production of these biocontrol agents have been evolved and popularized in the field stations.

#### Formulation of artificial diet for young age silkworm

Using different combinations of locally available inexpensive ingredients along with dry mulberry leaf powder, semi-synthetic artificial diets were formulated. These diets are suitable for young age silkworms up to 2<sup>nd</sup> instar. The growth, survival and larval duration of the batches fed on diet was found to be on par with that of the mulberry leaf fed larvae. Through artificial diet rearing, supply of better and balanced nutrients, maintenance of proper hygiene during young age silkworm rearing and reduction of labour cost could be achieved.

#### Phytoecdysteroids for uniform maturation

The search for a feasible technology by which the spinning activity in silkworm can be regulated is being explored. Recent studies have established that judicious administration of insect moulting hormone, ecdysterone can accelerate the maturation events and synchronise the spinning activities in silkworm. Ecdysteroids from locally available plants have been extracted and isolated. These phytoecdysteroids on administration to silkworm on the onset of spinning have been shown to induce uniform maturation and the process of mounting could be completed within 18-24 h as against 72 h in the normal course of mounting.

#### Molecular marker assisted breeding

Recent advances in plant and animal breeding have highlighted the prospects of using linked molecular markers (isozyme/DNA) for improvements of desirable traits. In silkworm, a number of biochemical parameters have been analysed in India and also elsewhere, which have yielded information indicative of their utility as markers in silkworm breeding for improving commercial traits.

Digestive amylase has been identified as a useful marker for improving the viability in silkworm because of its role in better digestibility and close association with survival. Distinct genetic divergence was also observed among silkworm stocks in terms of activity as well as isozyme polymorphism for cathodic amylase. All high yielding bivoltines were of the "Null" type with low activity and the low yielding high survival polyvoltine stocks were either of "4 band / 5" band types with high activity (Patnaik and Datta, 1995). A back cross breeding scheme was adopted and the "4 band / 5" band isozymes were introgressed into the genetic background of the high yielding CSR breeds used as recurrent parents. The evolved near isogenic lines of the CSR breeds have shown significant improvement in survival, at the same time, without any deterioration in productivity traits, which has been confirmed by on-farm trials conducted at field stations.

## Analysis of QTLs for cocoon traits using conventional genetic markers

Most of the economic traits are controlled by multiple (poly) genes, recently designated as Quantitative Trait Loci (QTL), whose expression is further modified by the environment. Until recently, the accurate estimation of the number, location and effects of these QTLs was a difficult task for the geneticists and breeders. Of late, mapping and analysis of QTLs has been possible with the aid of linked conventional or molecular genetic markers and also improved statistical methods (Patterson *et al.*, 1988;Tanksley *et al.*, 1982; Tanksley and Nelson,1996).

The Institute is having a collection of silkworm stocks carrying representative markers for 22 of the 28 linkage groups in silkworm. Using these stocks, as low yielding parents and high yielding wild type parents, back cross analysis was carried out. The results have shown major effects of 11 linkage groups for cocoon weight, shell

weight and shell ratio and seven linkage groups having significant effects on filament length and denier (Ashwath *et al.*, 1998).

### Isolation and purification of anti-bacterial and antiviral proteins in silkworm

Insects have efficient self defense mechanisms like phagocytosis, encapsulation and humoral responses against bacterial infections. The humoral responses mainly involved production of a variety of anti-bacterial substances like cecropins, attacins, defensins *etc.* (Boman and Hultmark, 1987). An induced anti-bacterial protein was isolated and purified from the hymolymph of the mulberry silkworm infected with *E. coli.* The purified protein was a single polypeptide chain of 16 kDa. The 20 N terminal amino acid sequence of the protein was determined and this sequence showed homology with those of lysozymes reported in other species (Abraham *et al.*, 1995).

It has been shown that anti-viral substances in the gut juice and Viral Inhibitary Factors (VIF) are produced in the hymolymph against BmNPV (Aizawa, 1991). Further, the Red Flourescent Protein (RFP) and alkaline proteases in the gut juice show anti-viral activity (Funakoshi and Aizawa, 1989). Two anti-viral proteins of 28 kDa and 66 kDa have been isolated, purified and characterized. The amino acid sequence has been determined and back translated into DNA using GCG programme. The sequence data will be used to design up and down primers for amplification of DNA and further screening.

# Monitoring of differential tolerance to BmNPV by luciferase assay

Work carried out earlier by screening 72 silkworm stocks through oral inoculation of BmNPV had revealed that the tolerance level of Indian non-diapausing strains to BmNPV was much higher than that of the diapausing strains of temperate origin. Similar observations were found in Chinese and Japanese reports.

With the objective of understanding the basis of differential response to BmNPV and also to elucidate the damage due to infection within the host, we used a recombinant BmNPV (vBmUW1luc) containing the firely luciferase reporter gene under the control of the p10 promoter of AcNPV. The gene expressed early during infection both *in vivo* and *in vitro* and led to synthesis of large quantities of luciferase which resulted in luminescence emission instantaneously on administration of the substrate luciferin. Nistari, an indigenous traditional non-diapausing strain which has many fold higher tolerance (LC<sub>50</sub> -  $7 \times 10^7$  PIB/ml) to BmNPV than the susceptible strain, Hosa Mysore (LC<sub>50</sub> -  $2 \times 10^6$  PIB/ml) which is an evolved non-diapausing type, were used for the study. The results

clearly demonstrated that the tolerance to BmNPV is primarily dependent on the route of infection and midgut may act as a barrier for the entry of the virus into the haemolymph and other body tissues preventing further multiplication of the virus and thus leading to resistance in Nistari. The observations suggest the possibilities of interaction of BmNPV with the midgut cell receptors and cellular humoral system. Thus, the use of recombinant NPV harbouring the luciferase reporter gene could be an ideal technique for monitoring the progression of viral multiplication and its quantification. This also can help as a regular tool to the silkworm breeders for screening the status of NPV tolerance and discriminating the divergent strains leading to the development of robust silkworm strains for tropics or elsewhere. The proposed protocol holds enormous scope for wide applications in basic as well as applied research in viral pathogenesis in general and BmNPV in particular.

### Development of immunodiagnostic kits for the early detection of silkworm diseases

Immunodiagnostic methods, *viz.*, Latex agglutination tests, enzyme based and colloidal dye based dipstic immunoassay have been developed for the detection of pebrine, NPV and IFV in silkworm (Nataraju *et al.*, 1994). The agglutination assay, enzyme based immunosorbant assay and enzyme based dipstick immunoassay for pebrine diseases were sensitive to detect 10 ng of spore surface protein and 400 spores/ml. The progressive infection could be detected within 2 days post-infection. The assays for NPV and IFV could detect 5-10 ng/ml of antigen. The progressive infection could be detected within 12 h post-infection. The kits developed will be of great importance in detection of the said diseases in seed production centers and young age rearing centers.

### Detection of RFLP markers linked to cocoon shell ratio

RFLP analysis of the cocoon shell ratio which primarily determines the silk yield was carried out. Inbred lines of silkworm stocks showing large differences for cocoon traits, *viz.*, B20A (high) and C.Nichi (low) were developed. On screening of low copy probes prepared from the genomic library, 37 revealed distinct polymorphism between the parents. For identification of RFLP markers, closely linked to the cocoon shell character, both bulked segregant analysis as well as near isogenic lines were used. The study has identified six RFLP markers linked to high shell (HSR) and 2 markers for the low shell ratio (LSR) (Table 7). The results suggest the prospects of using these markers as molecular tags in silkworm breeding for detection and selection of the target trait.

**Table 7.** Results of bulked segregant analysis with RFLP markers

Probe No.	Base Pair (kb)	B20A	C.Nichi	F1	F2 bulk HSR LSR
S-90	1.8	+	-	+	+-
S-96	1.6	+	-	+	+
S-119	1.2	+	-	+	+
S-350	1.6	+	-	+	+
S-501	1.7	+	-	+	+
S-566	0.5	+	-	+	+
S-117	0.4	-	+	+	- +
S-188	1.1	-	+	+	- +

### Genotyping of silkworm strains using molecular markers

Presently, DNA marker based approach is being widely used for tagging many traits of commercial importance. The first step in this direction is to develop a molecular map. Over the last few years, genome analysis of silkworm is being carried out using molecular markers. RFLP analysis of the silkworm strains of varied geographical origin and yield status have shown high levels of DNA polymorphism. Similarly, PCR based RAPD and also mini satellite markers were used to study DNA profiling of silkworm genotypes (Nagaraja and Nagaraju, 1995; Nagaraju et al., 1995). Recently, genetic characterization by simple sequence repeats (SSR)- anchored PCR has been taken up (Reddy et al., 1999) Each genotype revealed distinct and unique DNA pattern specific to diapausing and non-diapausing strains. The study indicated their potential use not only in understanding genetic relationships but also as powerful tools to generate markers that are linked to traits of interest in silkworm.

The impact of R & D in Indian sericulture is clearly evident by the phenomenal growth in silk production achieved since the 60s. This impressive progress is primarily due to the breakthroughs achieved both in the conventional as well as biotechnological strategies. Due to rapid industrialization and increased labour costs, the silk production is gradually decreasing in sericulturally advanced countries like Japan, China etc. Being the second largest producer of silk in the world today, India could be the key player in the production and trade of silk in the coming years. India has a daunting task ahead to fill the shortfall in global silk production to meet the world demand in addition to the domestic demand which is increasing year after year. To compete in the global market, India has to increase not only the quantity but also the quality of raw silk to international grade. The present

technologies so far developed if used as a package, will significantly increase the production of bivoltine silk which in turn will improve the quality. Hence R & D in sericulture in India has a pivotal role to play to boost the bivoltine silk production in the shortest possible time by redesigning the conventional strategies adopted so far supplemented with the new tools of biotechnology.

### Acknowledgment

The author acknowledges the help of S. K. Ashwath for compilation of data and preparation of the paper. Thanks are also due to S. Sekar for typing the manuscript.

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