PROCEEDINGS

ORGANIZATION

INTERNATIONAL SERICULTURAL COMMISSION

GREEK NATIONAL AGRICULTURAL RESEARCH FOUNDATION

GREEK MINISTRY OF RURAL DEVELOPMENT AND FOOD

Υπουργείο Αγροτικής Ανάπτυξης & Τροφίμων
ministry of rural development and food

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Speech of Dr Gerard Chavancy, General Secretary of the International Sericultural Commission.

Mr Chairman

Dear Presidents of sections

Dear Delegates

It is with great pleasure that I am talking to you today.

If we are gathered for this 21st Congress in Athens, it is thanks to the Greek authorities who have invited us and have taken care of the organization of this meeting. I would like to vividly thank them. And this all the more because the ISC Congress has not been held in Europe for a very long time. The last one was held in Versailles (France) in 1970.

This is maybe a sign that some European countries are involving themselves in a renewal of sericulture. In any case, I hope, that it is a positive sign in that sense.

As a result of the general economic situation, the number of participants in our XXIst Congress is not as high as we had estimated. However, these difficulties are made up by the fact that numerous new participants are present and that a number of papers as high as ever will be presented.

This congress is also the opportunity to open up a new section entitled “Sericulture in non-textile industries”. For this first issue, there are not many papers presented but, obviously, this section looks likely to expand by the next Congress.

I am sure that, thanks to our hosts I thank again, we will have an interesting meeting in the next 4 days and that interesting contacts will be

taken which will lead, I hope, to new collaborations between the different countries.

I wish you all an excellent Congress.

Dr Gerard Chavancy
General Secretary of the International Sericultural Commission.

International Sericultural Commission

2008 LUIS PASTEUR PRIZE

Dr S. B. DANDIN

Dr Dandin's 30 years of career have been dedicated to the development of sericulture. His career took place in two very important sericultural institutes: the Karnataka State Sericulture Research and Development Institute in Bangalore and the CSR&TI, from the Central Silk Board in Mysore.

He conducted several types of activities at the same time. First of all, researches in genetics and breeding through which numerous new mulberry and silkworm varieties, exploitable in sericulture, could be obtained.

New methods and new materials for mulberry and silkworm management were also developed by him. His work lead to the release of several patents.

Finally, a great part of his activity was dedicated to students' and sericulturists' training and to the popularization of scientific work.

The entire results and activities produced by Dr Dandin have contributed in a notable way to the development of sericulture in India and elsewhere.

It is logical that today, his major contribution to sericulture science and silk industry be rewarded by the Louis Pasteur Prize.

It is with great pleasure that I am remitting him this Prize today, because I have been able to appreciate Dr Dandin's professional and human qualities, for more than 20 years.

International Sericultural Commission

2008 PASTEUR PRIZE

Dr Toshiki TAMURA

Dr Tamura's researches have been dedicated, since the beginning of his career, to silkworms molecular biology and molecular genetics.

He has obtained numerous very important results published in international journals of high standard.

The achievement of transgenic silkworms thanks to the use of pyggyBac transposon was without any doubt, the most important of these results. The possibility of transforming the *Bombyx mori* silkworm has opened a completely new wide scope of either fundamental or applied researches. This work has earned him numerous national awards.

The foremost role of silkworm as a biological model has thus been confirmed and it has become a major biotechnological tool.

In fact, Dr Tamura has multiplied work related to the use of *B. mori* as a bioreactor by obtaining, for instance, the production of human pro-collagen. But numerous techniques enabling the improvement and the extension of the applications of transgenesis in the field of sericulture and of biomedicine were also developed by him.

I have known Dr Tamura for many years during which, we collaborated notably on the development of the genetic transformation of silkworm.

I have always valued his work capacity, the quality of his scientific processes and his opening of mind.

That is why I am very happy today to remit the Louis Pasteur Prize to Toshiki Tamura, whom I am considering as a friend.

ORAL PRESENTATIONS

A. MULBERRY SECTION

Mulberry Foliage Productivity for Sustainable Sericulture.

B.N. Susheelamma and Rekha M. Central Sericultural Research and Training Institute, Mysore – 570 008, India.

SUMMARY

In 21st century sustainability in silk productivity is declining. Therefore, effective utilization of natural resources and utilization of neem compost was carried out to obtain nutritive mulberry foliage productivity. The integrated mulberry cropping resulted in nutritionally efficient foliage productivity. This resulted in sustainable silkworm productivity and helped farmers to have economic viability.

INTRODUCTION

Sericulture is deep rooted in the culture and tradition of Asian countries and has been considered as an economically viable agro-industry in the rural areas. Keeping in pace with the living standards all over the world, the demand for silk is bound to increase in the International market. To meet this demand, there is dire need to take up intensive field research for qualitative and quantitative improvement in silk production with the emphasis on cost of silk productivity.

More emphasis has to be laid on cost of mulberry foliage productivity, since 60% of cost of silk productivity is incurred on cultivation of mulberry. Hence, productivity and profitability in sericulture depends mainly on maximization of foliage productivity per unit area.

Ravishankar et al (2002) have emphasized the role of efficient management of plant nutrients for effective water conservation in agriculture crops. Therefore, in mulberry efficient management of nutrition is very much required in 21st century to reduce irrigation level.

The soil biological management is very important to improve mulberry productivity. The silkworm (Bombyx mori. L) is responsible for the cocoon productivity and in turn to produce good quality silk. The health, growth and quality of the silkworm solely depend upon the nutritional quality of mulberry leaves which are fed to silkworm (Mala V.Rajan et al ; 2001).

The mulberry leaves best relished and utilized by silkworm are the ones which contain more moisture retention capacity for longer duration with high chlorophyll content and this has been expressed also by direct relationship of important silkworm productivity characters like shell weight, shell ratio, filament length, raw silk percentage and denier of silkworm (Susheelamm et al; 2006). No doubt that the nutritional quality of mulberry foliage plays a vital role in silkworm productivity. The leaf thickness and photosynthetic rate are found to be related to moisture conservation and net assimilation rate which contribute to the quality of leaves (Hesketh et al; 1985 and Susheelamma et al.; 2006). The important physio-anatomical characters are having direct role on nutritional efficiency of mulberry foliage. In many horticultural and grain crops neem resources are utilized extensively to control diseases, pests and

insects. (Nandagopal et al; 1990, Srivatsava et al; 1994, Udaiyan and Ramarathinam, 1994 and Narwal et al;1997). The botanical name of neem is Azadirachta-indica A. Juss.
In 21st century sustainability in silk productivity is declining. Therefore, following nutritionally efficient technologies to be adopted with utmost importance to improve mulberry foliage productivity in farmers fields.

1) Effective utilization of naturalresources in mulberry cultivation
2) Integrated cropping with other crops
3) Increasing the efficiency of water use and water delivery by plants
4) Utilisation of neem compost to control various foliar and root diseases
5) Qualitative foliage productivity with high chlorophyll content
6) Management of environment
7) Minimum usage of fungicides and pesticides to check environment pollution
8) Soil fertility management

METHODOLOGY

Neem trees were cultivated surrounding mulberry garden and shoot of neem trees was composted in compost pit. Green manure crop was cultivated as intercrop. Leafy vegetables were cultivated during rainy season along with mulberry and root stocks were mulched. Biofertilizer was utilized at the rate of 20 kg/ha/year in four split doses. Data pertaining to mulberry foliage productivity was recorded in progressive farmers fields during 2005-2008 from south India. Silkworm productivity data was collected for three years from the same farmers.

RESULTS AND DISCUSSION

The path analysis between important physiological and anatomical characters was worked out to know the nutritionally efficient characters of the foliage (Table-1). The photosynthetic rate is important nutritional character of leaf. The direct association between photosynthetic rate, moisture retention capacity and leaf thickness was observed (Table-1) and this indicates nutritionally efficient characters are responsible for silkworm productivity and in turn for sustainable sericulture.

The figure 1 and 2 depict the mulberry foliage productivity characters and silkworm productivity characters for three years (2005-2008) in the progressive farmers fields.
The leaf productivity was enhanced due to adoption of nutritionally efficient technologies. The neem compost helps to control foliar diseases like tukra, leaf roller, leaf spot and root rot. Control of foliar diseases enhances the foliage productivity. Neem is used as fungicide pesticide in many horticultural crops and developed countries like U.S.A. and Canada are producing neem compost in large scale and it is sold in market. The neem compost will build soil organic matter and acts as antibiotic to non-beneficial bacteria present in the soil. The inter cropping with green manure crop and leafy vegetables will supply nitrogen to mulberry plants and there by inorganic fertilizer cost is reduced and quantity of inorganic fertilizer used is reduced to 30% of recommended dosage. If the sericulture industry has to grow in Asian and

European countries, the soil fertility management has to be taken care to improve mulberry productivity, which will make sericulture sustainable.

To help farmers to have economically viable sericulture it is better to produce highly productive mulberry foliage that are nutritionally efficient. The thick succulent leaves with high chlorophyll content will remain in fresh condition for longer duration in the rearing house and to have sustainable sericulture moisture retention capacity of excised leaves is very important character contributing to mulberry productivity.

CONCLUSION

The present study enumerates the following findings:

- For better mulberry productivity, natural resources are to be utilized to maximum extent.
- Water management in mulberry fields to be given more importance.
- Cost effective organic resources like neem leaf compost, bio-fertilizer and green manuring are to be adopted in mulberry foliage production in the farmers fields.
- The application of pesticides and fungicides needs to be minimized or completely stopped.
- Keeping quality of foliage in rearing house is closely associated with thick foliage with high moisture content and moisture retention capacity. The high photosynthetic rate of foliage is also associated with thick leaf and which in turn reduces the transpiration from foliage.

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Table 1: Path analysis showing the direct and indirect effect of physio-anatomical characters on leaf thickness in mulberry.

Characters	Photosynthetic rate (μ mol cm^{-2} s^{-1})	Transpiration rate (μ mol cm^{-2} s^{-1})	Moisture retention capacity (%)	Correlation with leaf thickness (μm)
Photosynthetic rate (μ mol cm^{-2} s^{-1})	2.138	0.345	-0.424	0.795*
Transpiration rate (μ mol cm^{-2} s^{-1})	-1.936	-0.381	0.489	-0.906**
Moisture retention capacity (%)	1.649	0.339	-0.550	0.907**

Fig.1: Mulberry productivity in farmers fields during 2005-2008 with adoption of nutritional efficient technologies (average of 50 farmers)

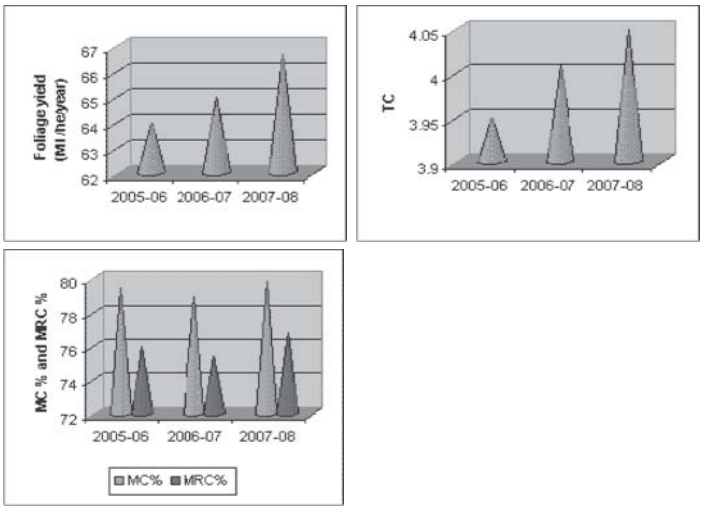
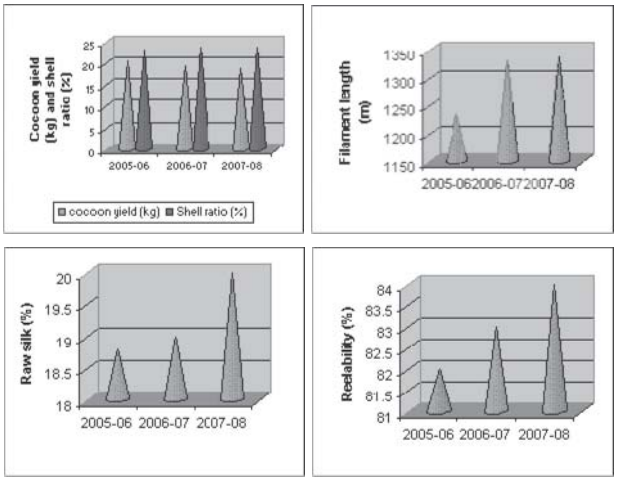


Fig.2: Silkworm productivity with farmers (race CSR2 x CSR4) fields with adoption of nutritional efficient technologies



Relationship between Planting System and Pruning Method on Mulberry Fruit Yield.

Sathaporn Wongareonwanakij[1], Wiroje Kaewruang[2]

1. **Queen Sirikit Sericulture Center (Udon Thani), Thailand.**
2. **The Queen Sirikit Institute of Sericulture, Ministry of Agriculture and Cooperatives , Thailand.**

Abstract

Besides of mulberry' s foliage being used as food for silkworm the mulberry fruits have also been used as food and beverage for human, such as mulberry fruit juice, wine and jam at the domestic and industry levels. Hence, a large number of mulberry fruits are needed to be raw materials which are normally not sufficient. Thereby, the research work on the increasing of the mulberry fruit yields must be considered. This experiment was studied on the comparative relationship between planting systems and pruning methods on the mulberry fruit yields var. Chiangmai. The experimental design was 2x3 factorial in RCB with 4 replications. The treatments were composed of 2 main factors. The first was the planting systems as row planting with spacing 0.75 x 2 meters and square planting with spacing 2 x 2 meters. The second was the pruning methods as 1). Single stem (top cutting at 1 meter above the ground with single stem) 2). High pruning (top cutting at 1 meter above the ground with 3 – 5 stems from the ground) 3). Low pruning (top cutting at 30 centimeters above the ground). The results indicated that the plants grown in the row planting system produced more yields than those grown in square planting. System. Also, the plants pruned with the high pruning produced the highest fruit yields compared to the other treatments. However, there was no significant interaction between the 2 main factors.

Introduction

A number of researches have been well established on the mulberry fruit modifications such as mulberry fruit juice and wine (Wiroje *et al.,* 1992) and jam (Wiroje *et al.,* 1995). Presently, these products have been increasingly produced at the domestic and industry levels, in particular mulberry fruit juice and mulberry wine products . There for it seems that this kind of fruit is one of the economic agricultural productions based of the country. Consequently , the present study was conducted on the comparative study on relationship between the planting system and the pruning method on the fruit yield of mulberry var.Chiangmai. The objective was to obtain the appropriate cultivation method, which was attributed to increase high yield per area and convenient cultivation especially during harvesting.

Materials and Methods

Mulberry plant var. Chiangmai was used. The experimental design was 2x3 factorial in RCB with 4 replications. The treatments comprised 2 main factors. The first was planting systems as row planting with spacing 0.75 x 2 meters

and square planting with spacing 2 x 2 meters. The second was pruning method as 1). Single stem (top cutting at 1 meter above the ground with single stem), 2). High pruning (top cutting at 1 meter above the ground with 3-5 stems from the ground) ,3). Low pruning (top cutting at 30 centimeters above the ground).After one year of growing during the early rainy season, the pruning methods as previously mentioned were operated. Defoliation occurred during early winter (November) and finished in December. The flowers and the leaf buds were sprouted at the same time as soon as they obtained the first rain of the early summer (late January –February). The yields were harvested in March until April. Thereafter, the plants were trained again by being cut at 20 cm away from the previous cutting point in all treatments. The farmyard manure was basal applied at the rate of 3 ton/rai/year. The chemical fertilizer grade 15-15-15 of N-P_2O_5-K_2O was also applied at the rate of 100 kg/rai/year. The water was supplied when it was necessary, to prevent flower or fruit dropping.

Results and Discussion

There were no interaction differences between the planting systems and the pruning methods (Table 1). Nonetheless, there were significantly differencewithin the factors on growth and yields of mulberry plants. The plants grownas the row planting system produced the higher yields than those grown as the square system did. The results were indicated that the 2 years old produccd the yield of 1,314 and 911 kg/rai in the row and the square planting systems, respectively. Whereas of the 3 years old plants produced the yields as 1,094 and 811 kg/rai in the row and the square planting systems respectively (Table 1). Although, the yields were not significant differences between the row and the square planting systems of the 4 years old plants, Wasan *et al*., (2002) reported that the planting systems had not affected on the yields of the mulberry plants. The reasons for that probably were differences in branch cutting methods and the quality of the original rootstocks. The present study indicated that the high pruning method caused the plants producing highly yields as 1,231, 1,077, and 4,274 kg/rai/year of the 2, 3 and 4 years old plants, respectively. Whilst the other two methods, the yields were not consistency. For examples, the 3 years old plants with the single stem pruning method produced the lowest yields as 700 kg/rai; whereas, the 4 years old plants with the low pruning method produced as 3,558 kg/rai which was lower than other methods.

The main factor on the growth of mulberry plant was the number of branches per plant(Table 2). The results indicated that the high pruning method caused the plant increasing branches higher than any other method as 10.4, 18, and 22 branches/plant at 2,3 and 4 years old plants, respectively. Subsequently, this method was attributed to the mulberry plants producing highly yield. For the planting systems, the plants grown with square planting system gave significantly higher branches than those grown as row planting system. However, in comparison with yield per planting area the plants grown with row systems produced higher than square plantings. That because the number of plantings /area was greater.

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Table 1 Comparison on fruit-yield of mulberry Var. Chiang mai (Kg/rai) on planting system in different pruning method (2-4 years old) at Queen Sirikit

Sericulture Center (Udon Thani)

Pruning method	2 years old			3 years old			4 years old		
	Planting system		Average	Planting system		average	Planting system		average
	row	square	Pruning method [1/]	row	square	Pruning method [1/]	row	square	Pruning method [1/]
Single stem	1,345	932	1,138 a	799	600	700b	4,700	4,142	4,421a
High pruning	1,360	1,102	1,231a	1,234	921	1,077a	4,086	4,462	4,274a
Low pruning	1,236	698	967a	1,248	912	1,080a	3,475	3,640	3,558b
Average	1,314	911 (**) [2/]		1,094	811(*) [2/]		4,087	4,081 (ns) [2/]	
Planting system									
C V. (%)	18.6			27.5			12.0		

Note [1/] Mean in a column followed by a common letter are not significant different at the 5% level by DMRT

[2/] (ns) not significant

(*) significant at 5% level

(**) significant at 1% level

Table 2 Comparison on branch number (No. branch/plant) during harvesting period of mulberry Var. Chiangmai on planting system in different pruning method
(2-4 years old) at Queen Sirikit Sericulture Center (Udon Thani)

Pruning method	2 years old			3 years old			4 years old		
	Planting system		Average	Planting system		average	Planting system		average
	row	square	Pruning method [1/]	row	square	Pruning method [1/]	row	square	Pruning method [1/]
Single stem	4.9	6.8	5.9 b	9.4	9.6	9.5b	13.4	19.8	16.6b
High pruning	8.7	12.2	10.4 a	14.1	22.0	18.0a	15.4	28.7	22.0 a
Low pruning	5.8	6.4	6.2 b	8.3	8.6	8.5b	12.0	15.4	13.7b
Average	6.5	8.5(**) [2/]		10.6	13.4(*) [2/]		13.6	21.3 (**) [2/]	
Planting system									
C V. (%)	22.2			23.1			29.1		

Note [1/] Mean in a column followed by a common letter are not significant different at the 5% level by DMRT

[2/] (*) significant at 5% level

(**) significant at 1% level

Phenolic content of leaves of different mulberry cultivars affect growth in the silkworm.

M.P. Germanς[a,] , V. D'Angelo[a], S. Catania[b], T.C. Miano[a], V. Perna[a], S. Faragς[c], L. Cappellozza[d], S. Cappellozza[d]

([a]Pharmaco-Biological Department, School of Pharmacy, University of Messina Vill. SS. Annunziata, 98168 Messina, Italy [b]Interdipartimental Center for Experimental Toxicology (CITSAL), School of Medicine, University of Messina, Italy, [c]Stazione Sperimentale per la Seta, Via Colombo 83, Milano, Italy, [d]Council of Research and Experiment in Agriculture (CRA), Apiculture and sericulture unit of Bologna, Padua seat, Italy).

1. Introduction

Mulberry trees have been cultivated for a long time in order to obtain the leaves to feed silkworms (*Bombyx mori* L.)

A screening of the most popular mulberry cultivars employed in sericulture in Italy was carried out, in order to test their leaf attitude to be used as an ingredient for silkworm artificial diet. Thus, in the present study, methanolic extracts from the leaves of different mulberry cultivars were screened for their phenolic content, identification and quantitative determination of some phenolic constituents.

2. Materials and Methods

2.1. Plant material

Samples of leaves collected during the spring season (May) from different cultivars (cvs) of *Morus* genus were provided by the Api-sericulture Unit, Bologna, Padua seat (CRA).

2.2. Extraction procedure

Leaf samples collected from different cvs underwent a lyophilization process immediately after harvesting. Afterwards, 10 g were extracted with 100 ml of a mixture of methanol and water (85:15, v/v) under a sonicator for 1 h. Then, the extracts were filtered and concentrated to dryness under vacuum .

2.3. Quantification of total phenols

An aliquot (0.1 ml) of sample solution (1 mg/ml) was mixed with 0.2 ml of Folin-Ciocoltau reagent (Analyticals, Carlo Erba), 2 ml of H_2O and 1 ml of 15% Na_2CO_3. After 2 h of incubation at room temperature in the dark, the absorbance was measured at 765 nm with a Shimadzu UV-1601 spectrophotometer and compared to a gallic acid calibration curve. Total phenols were expressed as gallic acid Equivalent per gram of extract (mg/g).

2.4. HPLC analysis

Rutin and phenolic acids were determined by high-performance liquid chromatography (HPLC) using a HP 1100 chromatograph equipped with a degaser, binary pump autosampler column oven and diode array detector. The results were expressed as milligrams per gram of extract.

2.5. Experimental insects
A silkworm two-way hybrid (118 x 120) (Japanese female) and a four-way hybrid (70x71) (120x125) (Japanese female) were used in order to test the growth of first instar larvae. Both hybrids were produced by the Apiculture-Sericulture Unit of CRA, Padua seat.

2.6. Artificial diet
The artificial diet used in the current experiment was patented by CRA. Seven different lots of artificial diet were prepared without mulberry leaf powder and six out seven were added, during cooling, which followed the sterilisation process, with a different phenol mixture; the seventh one was not added with phenols as it represented the control diet. For the test, 100 g of dry diet were mixed with amounts of extracts corresponding to 5 g of lyophilised leaves collected from the cultivars Florio, Ichinose, Korin, Morettiana, Okaraguwa and Spagna black fruit.

2.7. In vivo test
Two hybrids were fed on the different artificial diets from hatching to the completion of the first instar (end of the first moult). Three replicates of 50 larvae each were established for all the tested varieties and control diet. Larvae which did not feed on the diet at all and died were also counted.

2.8. Statistical analysis
The experiments were carried out in triplicate and each experiment was repeated three times. The results were expressed as mean ± standard deviation (SD). For *in vivo* test, all the data underwent two-way ANOVA, Tukey's post-hoc test for comparison of means and regression analysis.

3. Results
3.1. Extract yields and total phenols
The extract yields and total phenols are reported in Table 1.

Table 1: Yield and total phenolic content of extracts from the leaves of mulberry cultivars

Cultivar	Yield (% w/w)	Gallic acid equivalent (mg/g)
Florio	11.13 ±4.03	76.08 ±1.14
Ichinose	10.08 ±0.56	103.03 ±2.12
Korin	15.23 ±0.02	110.94 ±0.82
Morettiana	13.76 ±2,19	71.45±2.05
Okaraguwa	12.86 ±1.48	118.67±3.23
Spagna black fruit	14.24 ±6.50	69.05±4.19

3.2. HPLC Analysis
The results of HPLC analysis are shown in Table 2.

The following compounds, chlorogenic acid, ferulic acid, gallic acid, sinapinic acid and rutin were detected in the extracts of mulberry cultivars. A low content of gallic acid was evidenced in the cultivars Florio, Ichinose, Korin and Morettiana. Moreover, the presence of sinapinic acid was found only in the cultivars Korin and Okaraguwa.

Table 2. The phenolic compounds isolated from the extracts of mulberry cultivars (μg/mg of extract).

Cultivar	Chlorogenic acid	Ferulic acid	Gallic acid	Sinapinic acid	Rutin
Florio	29.61 ±1.05	0.88 ±0.98	0.19 ±1.03	nd	5.94 ±1.98
Ichinose	58.20 ±2.03	0.48 ±0.05	0.28 ±0.89	nd	7.47 ±1.50
Korin	24.58 ±1.30	0.71 ±0.30	0.21 ±0.12	nd	0.10 ±0.6
Morettiana	24.06 ±2.01	1.14 ±0.50	0.29 ±0.17		
Okaraguwa	53.87 ±0.95	0.39 ±0.06	nd	3.62 ±1.02	15.95 ±0.09
Spagna black fruit	32.36 ±1.92	0.42 ±0.90	nd	nd	5.43 ±1.05

3.5. Growth of silkworm larvae

Two-way ANOVA analysis demonstrated that means are significantly different in the two hybrids among the various cvs ($P<0.05$). Furthermore, the interaction hybrid x cv is highly significant ($P<0.05$) (Table 3a, 3b, 3c). The coefficient of determination R^2, is 0.899, while the adjusted R^2 is 0.852.

Table 3a. Tukey's test (Hybrids)

Hybrids	Total number of larvae (mean + s.d.) moulted to II instar				
118 x 120	36.67 ± 8.11	A			
(70 x71)x(120x125)	23.10 ± 8.62		B		

Means followed by different letters are significantly different at $P < 0.05$ (Tukey's test).

Table 3b. Tukey's test (Mulberry cvs)

Cvs	Total number of larvae (mean + s.d.) moulted to II instar				
Florio	37.33 ± 3.78	A			
Korin	34.83 ± 3.37	A			
Okaraguwa	34.67 ± 11.18	A			
Morettiana	34.00 ± 12.15	A			
Spagna black fruit	24.50 ± 11.84		B		
Ichinose	24.33 ± 12.42		B		
Control	19.50 ± 3.73		B		

Means followed by different letters are significantly different at P < 0.05 (Tukey's test).

Table 3c. Tukey's test (interaction cvs x hybrids)

Cvs x hybrids	Total number of larvae (mean + s.d.) moulted to II instar				
Morettiana (118 x 120)	44.67 ±1.15	A			
Okaraguwa (118 x 120)	44.67 ±1.53	A			
Florio (118 x 120)	39.67 ±3.51	A			
Korin (118 x 120)	37.33 ±0.58	A			
Florio (70x71)x(120x125)	35.00 ±2.65	A	B		
Spagna black fruit (118 x 120)	34.67 ±4.51	A	B		
Ichinose (118 x 120)	33.67 ±8.02	A	B	C	
Korin (70x71)x(120x125)	32.33 ±3.05	A	B	C	
Okaraguwa (70 x71)x(120x125)	24.67 ±3.21		B	C	D
Morettiana (70 x71)x(120x125)	23.33 ±5.13		B	C	D
Control diet (118 x120)	22.00 ±3.00			C	D
Control diet (70 x71)x(120x125)	17.00 ±2.65				D
Ichinose (70 x71)x(120x125)	15.00 ±7.00				D
Spagna black fruit (70 x71)x(120x125)	14.33 ±4.51				D

Means followed by different letters are significantly different at P < 0.05 (Tukey's test).

Conclusion

In this study, a screening of the most popular mulberry cultivars was carried out, in order to test their phenolic extract attitude to be used as an ingredient for silkworm artificial diet. The present experiment, according to Kato (1978) confirms that phenols are indispensable components of the synthetic diet for this insect and enhance the rate of development in the early stages.

A remarkable finding of this research is that quality of the phenol mixture, more than quantity affects silkworm growth. The experiment demonstrated that phenolic extracts of the cvs Korin and Okaraguwa, characterised by an high phenolic content, are capable of sustaining silkworm growth in the first and more critical phase of development, similarly to other cvs traditionally employed in sericulture in Italy (Florio and Morettiana).

Integrated Nutrient Management through Bio – Inoculants. A component for higher Productivity in Rainfed Sericulture.

R. N. Bhaskar*, Wolktole sori S. Sarithakumari and K. R. Shashidhar, Department of Sericulture, UAS, GKVK, Bangalore-65, India.*Sericulture College, UAS, Chintamani.

ABSTRACT

An investigation was carried out on the influence of bio-inoculants on growth and yield and its subsequent effect on cocoon productivity of *Bombyx mori* L. (PM x CSR2) was revealed that, use of *Azotobacter* @ 25 kg/ha/yr, *A. awamori* @ 25 kg/ha/yr and *T. harzihanum* @ 20 kg/ha/yr (T1) had showed positive effect compared to standard check (100:50:50 kg/ha/yr NPK + 12 MT/ha/yr FYM), further it was also recorded maximum growth parameters *Viz*., No. of shoots / plant (28.00 & 27.45; 37.78 & 35.11), average shoot height (65.84 & 89.48; 71.04 & 94.74), plant height (1991.90 & 2600.51; 2479.53 & 3149.37), no. of leaves / shoots (12.92 & 17.23; 12.19 &14.41) and total no. of leaves / plant (361.38 & 380.47 ; 459.50 & 510.90) for crop I and crop II after 45 and 60 days after pruning, respectively. It was also further reflected in the fresh leaf yield per plant (403.60 & 538.13; 718.74 & 867.57) and leaf area index (11.38 & 18.79) at 60 days after pruning in I^{st} and II^{nd} crop, Further, the leaves harvested and fed to 4^{th} and 5^{th} instar larvae of PM x CSR2 recorded maximum cocoon yield of 640.88 & 691.11g / DFLs for I^{st} and II^{nd} crop followed by T4 (638.32 & 684.91g) and T2 (571.33 & 622.55 g/ DFLs) which were found to be superior over other treatment combinations.

Key Words: M_5 mulberry, bio-inoculants, growth parameters, productivity, rainfed condition

Introduction

Use of inorganic fertilizers have played a significant role in providing nutrients for highly nutrient intensive mulberry crop, which brought about manifold increases in mulberry yield. But it has been realized that, in the recent past, increase in yield was achieved at the expense of soil health. Moreover, some portions of the nutrients applied to the soil are still bound to be unused as they are not available to the plant. This increases the cost of production. The use of inorganic fertilizers is also becoming costlier from time to time. Further, with the application of inorganic fertilizers alone, particularly in unbalanced manner, problems such as diminishing soil productivity and multiple nutrient deficiencies appears (Krishna and Bongale, 2001). To this end complementing inorganic nutrients with bio-inoculants and FYM is a cost effective means to achieve the desired ends by overcoming the problems of soil degradation and poor leaf production in mulberry sericulture.

Material and Methods

Influence of bio-inoculants (*Azotobacter* sp. @ 20kg/ha/yr, *Aspergillus awamori* @ 25 kg/ha/yr and *Trichoderma hazianum* @ 20 kg/ha yr), FYM @ 12 MT/ha/yr and inorganic fertilizers (N-urea, P- single super phosphate and K- muriate of potash @100:50:50 and reduced doses plus bio-inoculants) were tested on M_5 mulberry growth parameters under rainfed condition at Gandhi Krishi Vignana Kendra, UAS, Bangalore in the Department of

Sericulture premises (77°35' E longitude and 12°58' N latitude at an altitude of 930 m above mean sea level). The bio-inoculants were obtained from bio-fertilizers scheme, Department of Agricultural Microbiology, UAS (B), GKVK. The bio-inoculants were mixed with FYM under wet condition and applied to the root zone of mulberry two weeks after the application of chemical fertilizers to avoid possible contact. The experiment was laid-out in a Randomized Complete Block Design (RCBD) with eight treatments each with three replications. A single plot size of 4m x 4m ($16m^2$) was used for each replication. A spacing of 60cm x 60cm between plants and rows were maintained for the experiment.

Notation	Treatments details
T_1	:Standard check: R. NPK* + R. FYM**
T_2	:75% NP through chemical fertilizers + 25% NP through *Aspergillus awamori* and *Azotobacter* sp. + R. FYM + R. K***
T_3	:50% NP through chemical fertilizers + 50% NP through *A. awamori* and *Azotobacter* sp. + R. FYM + R. K
T_4	:75% NP through chemical fertilizers + 25% NP through *A. awamori* , *Azotobacter* sp. and *Trichoderma hazianum* + R. FYM + R. K
T_5	:50% NP through chemical fertilizers + 50% NP through *A. awamori* , *Azotobacter* sp. and *T. hazianum* + R. FYM + R. K
T_6	:R. NPK only through FYM
T_7	:R. NPK only through chemical fertilizers
T_8	:Control: No application of any fertilizer

Note:*R. NPK: Recommended nitrogen, phosphorus and potash (100:50:50kg/ha/yr),
**R. FYM: Recommended farm yard manure (12 MT/ha/yr), ***R. K: Recommended Potash.

After one and two weeks of bottom pruning the chemical fertilizers and bio-inoculants along with FYM were applied, respectively as per the treatment details. Observations on growth parameters were recorded at 45 and 60 days after bottom pruning.

Results and discussion

The results of the study are presented (Table 1 and 2) and discussed in the light of the available literature. The response of mulberry variety M_5 to different bio-inoculants showed positive effect in terms of growth parameters during experimentation.

Number of shoots per plant

Non-significant differences were observed among the treatments with respect to number of shoots per plant in both the crops. However, maximum and minimum number of shoots per plant was observed for the plots that received recommended rate of fertilizer and control, respectively in both crops at 45^{th} and 60^{th} days after pruning (Table 1).

The improvement in the number of shoots per plant may be attributed to the effect of bio-inoculants and it is considered as complimentary functioning factor of bio-inoculants (*Azotobacter* sp., *Aspergillus awamori* and *Trichoderma harzianum*) with FYM and IF in terms of nitrogen fixation, P solubilization and in production of plant growth promoting substances.

Application of combined nutrients from different sources is believed to supplement the essential nutrients thereby augmenting mulberry growth.
Umakanth and Bhagyaraj (1998) have reported that, dual inoculation of nursery bed with *G. fasciculatum* and *Azotobacter chroococum* considerably increased plant growth and development of mulberry saplings. The present finding is further supported by the findings of Jayaraj *et al*. (2006).

Average shoot height (cm)
The shoot height of M_5 mulberry variety was significantly influenced by the application of bio-inoculants along with Inorganic Fertilizers (IF) and Farm Yard Manure (FYM). In the first crop, 45th and 60th days after application, maximum shoot height of 71.04 and 94.74 cm was recorded in T_1. This treatment was statistically found non-significant from T_4 (67.66 and 88.89 cm) and T_2 (63.43 and 84.57 cm). On the other hand, minimum shoot height of 53.48 and 71.31 cm was recorded in T_8 (control) 45th and 60th days after pruning. Similar trend was observed even in the second crop (Table 1).
The increased shoot height in both the crops may be due to the added effect of bio-inoculant: *Azotobacter* sp., which helped in fixing more atmospheric nitrogen. Further, addition of *A. awamori* and *T. harzianum*, a phosphorus solubilizing fungi has an added advantage in releasing the bounded form of phosphorus for nutrient use efficiency of mulberry leading to better growth and development. The results of the present study are in conformity with the findings of Nagarajan *et al.* (1986), who reported that, combined application of phosphorus solubilizing bacteria along with nitrogen fixers had a significant effect on increasing the plant height.

Number of leaves per shoot
The trend was even the same in the number of leaves per plant. However, in crop I, T_1 has recorded maximum number of leaves per shoot (12.92 and 17.23) 45th and 60th days after pruning, respectively which was followed by T_4 and T_2. On the contrary, the lowest number of leaves per shoot (8.06 and 10.74) was recorded in T_8 (Table 1). In crop II, the number of leaves per shoot did not significantly varied among the treatments experimented except for the control 45th days after pruning. Maximum numbers of leaves were recorded in T_1 (12.19 and 14.41) 60th days after pruning, which were on par with T_4 (11.67 and 13.74), T_2 (11.33 and 13.44) and T_5 (11.17 and 13.11). But minimum number of leaves per shoot for both the crops was recorded in control plots (Table 1).
Generally plants which received the three bio-inoculants in combination with recommended FYM and 25 per cent IF (NP) showed maximum number of leaves, similar to the plots that received full recommended dose of fertilizers, when compared to the sole application of IF or control which was attributed to activation of bioactive substances, resulting in increased availability of nutrients leading to more number of leaves in mulberry. Moreover, the increased number of leaves may be due to increased number of shoots per plant.
The efficacy of *Azotobacter* and *Azospirillum* for mulberry growth has been reported earlier (Nagarajan *et al*., 1986). The present investigation revealed the efficacy of the test bio-inoculants in improving the growth and development of mulberry by enhancing branching, plant height, number of

leaves per plant of mulberry. Similar findings were earlier observed by Gangwar and Thangavelu (1992).

Total number of leaves per plant

Influence of bio-inoculants along with IF and FYM in both the crops recorded significant results on total number of leaves per plant. In crop I, T_1 has recorded maximum number of leaves per plant (361.38) which was statistically significantly different from all the other treatments 45th days after pruning, it was followed by T_4 (292.02) and T_2 (278.15), respectively. 60th days after pruning, maximum number of leaves per plant was registered in T_1 (380.47), which was statistically on par with all the treatments except control. On the contrary, the lowest number of leaves per plant (212.98 and 258.90) was recorded in T_8 at 45 and 60 days after pruning, respectively. In crop II, maximum number of leaves per plant (459.50 and 510.90) was registered in T_1 which was on par with all the treatments except T_7 and T_8. Minimum number of leaves per plant in both the crops was recorded in the control at 45th and 60th days after pruning (Table 1).

Fresh leaf yield per plant (g/plant)

In the first and second crops, T_1 has recorded maximum fresh leaf yield per plant (403.60 and 538.13; 718.74 and 867.57 g/plant), which was consistently and statistically on par with T_4 (397.80 and 530.40; 614.07 and 795.20 g/plant) 45th and 60th days after pruning, respectively. Minimum fresh leaf yield (257.00 and 342.67; 226.77 and 280.05g/plant) was recorded for the control 45th and 60th days after pruning (Table 2).

The increased leaf yield may be due to more number of shoots, higher plant height and more number of leaves per plant in T_1 and T_4. Further, the combined application of FYM might have helped in slow and steady release of nutrients in addition to supply of important macro- and micro-nutrients besides efficient supply of N and P by nitrogen fixing and phosphorus solubilizing bio-inoculants utilized. Thus, NP application can be curtailed to the tune of 25 per cent by supplementing with bio-inoculants without adverse effect on fresh leaf yield of M_5 mulberry under rainfed condition. Katiyar *et al.* (1995) in their earlier studies on the effect of dual inoculation of *A. chroococum* and *G. mosseae* with 50 per cent recommended dose of N and P fertilizers have reported that, the yield of mulberry was on par with uninoculated control receiving full dose of N and P fertilizers. Further, confirmed by Das *et al*. (1994) and Sreeramulu *et al*. (2004) they also reported a saving of 25 per cent N and P chemical fertilizers application without affecting leaf yield of mulberry when *A. brasilense* and *B. megaterium* were inoculated.

Cocoon yield (g/dfl):

In the first and second crop, maximum cocoon yield of 640.88 g/dfl was registered in the batch of worm reared on mulberry leaves from T_1 (application of RNPK and RFYM), which was on par with T_4 (638.32 g/dfls) followed by T_2 (577.33 g/dfl), T_5 (548.56 g/dfl) and T_3 (520.89 g/df). The lowest cocoon yield (437.10 g/dfl) was recorded in control plot (Table 2). Favorably the beneficial effect of bio-inoculants can be envisaged from the present findings which clearly demonstrate the realization of not only mulberry growth, development and quality improvement on par with the standard check but also

the subsequent effect on growth, development and cocoon productivity of silkworm ($PMxCSR_2$).

Thus supplementation of bio-inoculants along with FYM and IF to mulberry can result to the required ultimate cocoon productivity without significant sacrifices under rainfed condition. Therefore, NP can be reduced to the tune of 25 per cent without affecting the silkworm, *B. mori* cocoon productivity. This is due to the fact that, the inoculation of bio-inoculants had resulted in availing the macro and micronutrients to mulberry root thereby maintaining the quality of mulberry leaves which provided all the required nutrients to the larvae in tune leading to good cocoons. In the long run, the benefit obtained from the microbes which are natural, renewable, eco-friendly and economical will be manifold in the present scenario of environmental hazards caused by indiscriminate use of chemical fertilizer to mulberry ecosystem. The present results are in close conformity with the finding of Umesh (1999) who opined the conjunctive use of *Azotobacter* and half the recommended dose of N had positive influence on cocoon and silk productivity in mulberry silkworm. An increase of 7.3 kg/100 dfl in cocoon yield was reported by Venkataramana *et al*. (2001) when tricontanol and *Azotobacter* were integrated in mulberry cultivation.

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Table 1: Influence of bio-inoculants on growth parameters of M_5 mulberry at 45 and 60 days after pruning

Treatments	No. shoots /plant				Average shoot height (cm)				No. leaves /shoot				No. leaves/plant			
	Crop I		Crop II		Crop I		Crop II		Crop I		Crop II		Crop I		Crop II	
	45DAP	60DAP	45DAP	60DAP	45DAP	60DAP	45DAP	60DAP	45DAP	60DAP	45DAP	60DAP	45DAP	60DAP	45DAP	60DAP
T_1	28.00	27.45	37.78	35.11	71.04	94.74	65.84	89.48	12.92	17.23	12.19	14.41	361.38	380.47	459.50	510.90
T_2	27.00	25.67	33.33	29.44	63.43	84.57	63.33	86.26	10.46	14.53	11.33	13.44	278.15	362.85	379.24	457.54
T_3	26.22	25.00	30.00	27.33	58.18	77.49	57.20	80.48	10.08	13.41	11.15	12.67	272.16	349.38	349.14	412.07
T_4	27.11	27.36	34.00	30.45	67.66	88.89	63.81	86.35	11.18	14.61	11.67	13.74	292.02	370.65	391.60	508.52
T_5	26.44	25.66	32.11	27.78	59.90	79.87	59.20	85.22	10.26	13.66	11.17	13.11	274.84	350.23	362.22	417.69
T_6	26.00	24.11	31.33	27.67	53.57	71.43	56.34	78.48	9.21	12.28	11.00	12.82	238.41	295.86	331.77	388.92
T_7	26.11	25.00	29.78	24.78	53.94	71.76	53.03	77.63	10.48	13.40	10.52	11.89	273.63	344.18	305.94	354.41
T_8	25.89	24.11	29.45	24.22	53.48	71.31	47.65	67.78	8.06	10.74	8.95	10.41	212.98	258.90	266.19	288.61
F-test	**NS**	**NS**	**NS**	**NS**	*	*	*	*	*	*	*	*	*	*	*	*
SEm±	**0.90**	**1.64**	**2.10**	**3.03**	**4.14**	**5.76**	**1.99**	**2.76**	**0.33**	**0.48**	**0.6992**	**0.495**	**12.82**	**30.15**	**30.404**	**51.133**
CD @ 5%	**-**	**-**	**-**	**-**	**12.55**	**17.47**	**6.02**	**8.38**	**0.99**	**1.47**	**2.1211**	**1.501**	**38.88**	**91.45**	**92.23**	**155.11**
CV (%)	**5.83**	**11.14**	**11.29**	**18.52**	**11.91**	**12.47**	**5.90**	**5.87**	**5.47**	**6.07**	**11.013**	**6.688**	**8.06**	**15.40**	**14.805**	**24.110**

Table2: Influence of bio-inoculants on fresh leaf yield/plant and cocoon yield (g/dfl)

Treatments	Fresh leaf yield/plant (g)				Cocoon yield (g/dfl)	
	Crop I		Crop II		Crop I	Crop II
	45DAP	60DAP	45DAP	60DAP	-	-
T_1	403.60	538.13	718.74	867.57	640.88	691.11
T_2	397.00	529.33	526.00	605.70	577.33	626.55
T_3	373.60	498.13	421.33	452.71	520.89	569.12
T_4	397.80	530.40	614.07	795.20	638.32	648.91
T_5	390.80	521.07	503.92	581.60	548.56	570.83
T_6	360.20	480.27	364.20	497.10	511.16	563.35
T_7	373.20	497.60	323.07	377.77	514.03	545.21
T_8	257.00	342.67	226.77	280.05	437.10	469.05
F-test	*	*	*	*	*	*
SEm±	**10.37**	**13.82**	**57.912**	**78.24**	6.97	4.78
CD @ 5%	**31.45**	**41.93**	**175.68**	**237.34**	21.15	14.55
CV (%)	**4.86**	**4.84**	**21.70**	**25.23**	2.20	1.40

Key: T_1- Standard check (RNPK + RFYM), T_2- 75 % NP through IF and 25 % through *A. awamori* and *Azotobacter* sp. + RFYM + RK T_3 – 50 % NP through IF and 50 % NP through *A. awamori* and *Azotobacter* sp. + RFYM + RK, T_4 - T_2 + *Trichoderma harzianum,* T_5 – T_3 + *Trichoderma harzianum,* T_6 – RNPK from FYM, T_7 – RNPK only from IF, T_8 –control.

Organic Sericulture for Bivoltine Production.

C. K. Kamble and B.N. Susheelamma, Central Sericultural Research and Training Institute, Mysore – 570008, India.

SUMMARY

Organic farming will help to improve mulberry foliage productivity and soil biological properties. Soil health is very important aspect to be taken care to get nutritious foliage for bivoltine silkworm productivity. The farmers who adopted organic sericulture were able to get good bivoltine production and farmers who did not adopt organic farming were not able to get good bivoltine productivity. In 21st century organic sericulture has immense value in bivoltine production.

INTRODUCTION

China has a history of over 5000 years for sericulture. Nearly 30 million farmers are involved in sericulture production in china. Cocoon production is about 500,000 tons per year and nearly 70% of the total production of the world. The organic farming is effectively practiced in china for mulberry foliage production, where as in other Asian countries including India inorganic farming has taken upper hand in mulberry foliage productivity in comparison to organic farming in farmers' fields. Therefore, sustainability in bivoltine silk production has not been achieved in India. Hardly 10% of total silk production is bivoltine in India.Organic sericulture has developed rapidly and practiced in China in farmers fields.

Organic sericulture helps for minimization of environmental pollution. Soil biological properties are enhanced and bivoltine silkworm crop losses are minimized. One of the effective ways of increasing the yield of cocoon per unit area of mulberry is to improve nutritive values of mulberry leaves. The higher the quality of the leaves fed to bivoltine silkworm, the lower is the quantity of leaves required by silkworm (Shablovsakaya and Kafian 1967). In present scenario of 21st century, the challenges before the sericulturist is how well he can manage the farm to enhance returns on a sustainable basis by way of increasing bivoltine productivity followed by improvement of economic efficiency.

Bongale and Dandin (1993) emphasized the effectiveness of nitrogen fixing bacterial biofertiliser in mulberry cultivation. Watanabe (1984) concluded in his research studies that use of green manure is very important aspect as a source of organic matter in rice cultivation. Therefore, sericulturists of Tamilnadu are using biofertiliser in more quantity as organic resource and cultivation of sunhemp as green manure to build up soil organic matter. Organic agriculture has developed rapidly in china and spread around the world (Biao, X and Xi aorong, W., 2003)

To meet the challenges of bivoltine production in Asian countries, organic sericulture has to be intensified in seri-productivity. Wide spread use of inorganic fertilizers has affected soil health and in turn affected the bivoltine production.

METHODOLOGY

Progressive sericulturists of South India were given technical guidance by spot demonstration method on adoption of organic sericulture. The data pertaining to mulberry productivity was recorded for three years from the sericulturists with organic and inorganic agri-practices.

RESULTS AND DISCUSSION

The data pertaining to mulberry and silkworm productivity are depicted in figures 1 to 6. The mulberry leaf productivity was higher in organic farmers and with out organic farming affected mulberry productivity. The foliar diseases of mulberry are completely controlled in organic farming fields. The organic farming foliage photosynthetic efficiency is better in comparison to inorganic foliage photosynthetic efficiency. Organic farming adopted by sericulturists got good bivoltine crop productivity and sericulturists with inorganic farming were affected by silkworm crop losses due to diseases. The use of biofertiliser and cultivation of green manure crop has helped sericulturists of Tamilnadu to improve soil health and organic carbon content. Due to this, sericulturists are able to produce high quality bivoltine silkworm productivity. Organic carbon content and nitrogen content of the soil play major role in production of nutritious leaf productivity. Therefore, organic sericulture will help to improve bivoltine silkworm productivity.

The present study enumerated important findings:

1. Organic sericulture has to be intensified in farmers fields to increase bivoltine production.
2. Organic sericulture is the best method to improve soil health.
3. Leaf photosynthetic efficiency has got direct role in bivoltine productivity.
4. To increase world bivoltine production, organic sericulture is the only best solution.

CONCLUSION

Organic matter is one of the most important constituents of the soil and from antiquity man has recognized its importance in regulating soil fertility at desirable levels. Organic sericulture is the only menas to achieve the sustainable target in bivoltine productivity in Asian countries.

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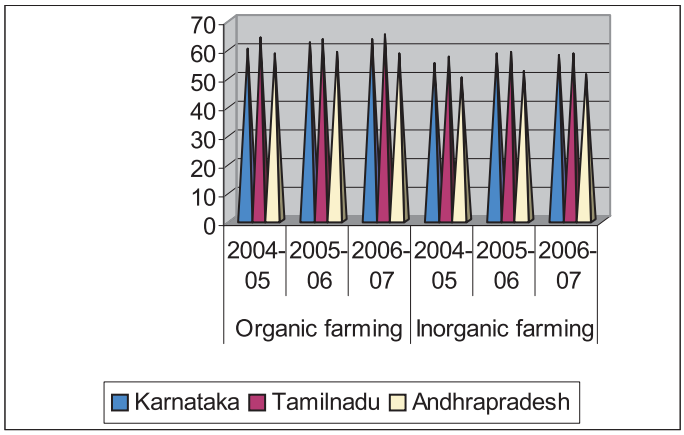
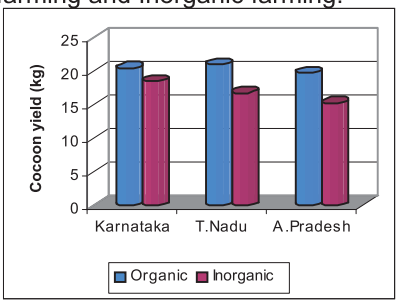
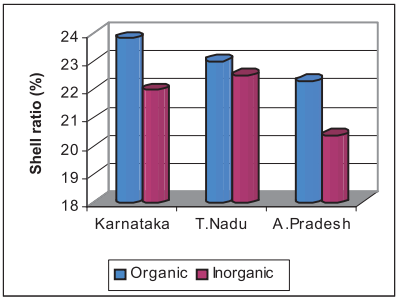
Fig. 1 : Foliage productivity in different states of south India with *Organic farming and **Inorganic farming. (Variety: V1)

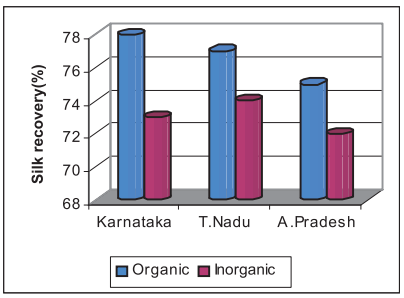


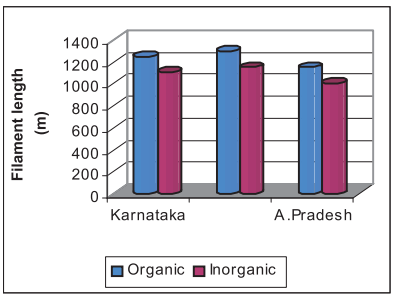
Note : *Organic farming – Leaf compost + Sunhemp as green manure crop + biofertiliser @20 kg/ha/year + 40 MT/ha/year organic manure + fertilizer @ 100:80:60 / ha/year.
** Inorganic farming – Present Central Silk Board recommendation – fertilizer @350:120:120 + 20 MT organic manure/ha/year.

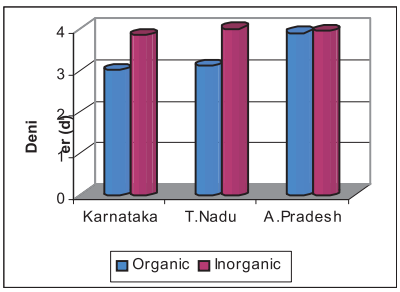
Fig.2: Silkworm productivity from farmers fields of South India with Organic farming and Inorganic farming.











Evaluation of *Morus laevigata* Wall. in *ex-situ* field gene bank

A.Tikader* and S.B. Dandin[1]
Central Sericultural Germplasm Resources Centre, Hosur -635 109, Tamil Nadu, India
National Silkworm Seed Organization, Bangalore - 560 068, Karnataka, India
***Corresponding authors e-mail: atikader_csgrc@yahoo.co.in**

ABSTRACT

The evaluation of *Morus laevigata* Wall. ex-Brandis. were carried out at Central Sericultural Germplasm Resources Centre, Hosur, India in *ex-situ* condition. Growth performance and leaf yield of mulberry accession studied, showed great extent of variation among eight growth traits. Different growth traits showed variation among the accessions. Number of shoot varied from 10.17 – 23.33 followed by length of the longest shoot (80.83 – 205.33cm), inter nodal distance (4.56 – 9.15cm), 100 leaf weight (381.73 – 1093.02g), total shoot length (572.67-3277.83cm), moisture content (68.97 – 73.30%), moisture retention capacity (61.11-83.62%) and leaf yield per plant (0.39 –3.00kg). Analysis of variance indicates significant variation at P<0.01 levels. The season x accession interaction was positive with all the accessions. The correlation study indicated that leaf yield was associated with number of shoot (0.51*); length of the longest length (0.67**) and total shoot length (0.67**). Besides this, other traits showed complex relationship among themselves both at phenotypic and genotypic level. Similar results observed in case of genotypic correlation but the magnitude was higher than simple correlation. The result indicates the performance of *M.laevigata* in *ex-situ* and its subsequent utilization for crop improvement.

Key words: Mulberry germplasm, evaluation, *ex – situ*, *M.laevigata.*

Introduction

In India *M. laevigata* Wall. is distributed throughout country and grows in different habitats ranging the altitude from 0 – 1500m above mean sea level. The wide distribution of mulberry indicates its adaptability and plasticity to various environments. Brandis (1906) and Hooker (1885) have reported 4 species viz., *Morus indica, M. alba, M. laevigata* and *M. serrata* occurring in India. Most of the Indian varieties belong to *M. indica and M. laevigata* is available in wild and cultivated forms. Mulberry is extensively cultivated for silkworm rearing in tropical, sub-tropical and temperate zones ranging from 50ĺ north latitude to 10ĺ south latitude (Yokayama, 1962). *In-situ* conservation of fauna and flora is though preferred but it is always not practicable and hence the most practical way is *ex- situ* conservation in field gene bank (Tikader and Rao, 2001). There are some reports on distribution and utilization of *M. laevigata* (Dandin *et al*., 1995; Ravindran *et al*., 1999, Tikader *et al*., 2002). But the field performance of *M. laevigata* is scanty after establishment in *ex-situ* field gene bank. The present study was undertaken to study the growth behaviour of wild and domesticated collections of *M. laevigata* to document the variation, association of different traits.

Materials and Methods

The present study was carried out at Central sericultural Germplasm resources Centre (CSGRC) Hosur during 2002 – 2005. Initially the material was collected through survey and exploration from different parts of the country and is being maintained at the field gene bank. The germplasm is being maintained at CSGRC, Hosur, India (Latitude 12.45′l N and Longitude 77.51′l E, altitude 942m above mean sea level under tropical climatic condition). The germplasm plantation was maintained under 2.44 x 2.44m with 4 plants per accession as small tree and randomly planted with control plants in Augmented Design. The maintenance of the germplasm followed as per the recommended cultural practices (Dandin and Sengupta, 1988; Tikader and Rao, 2001). When the plantation attained two years of establishment the plants were pruned at 1.5m crown height following two pruning per year for data recording. A total of 15 *M. laevigata* was subjected to detailed study at *ex-situ* field gene bank (Table 1).

Data on growth parameters were recorded after 90 days of pruning. Leaf moisture content and leaf moisture retention in harvested leaf was calculated following the procedure as reported earlier (Vijayan *et al*., 1997). The general statistics, analysis of variance, genetic estimates, correlation and divergence analysis was followed as per the statistical package SPSS.

Results and Discussion

The mean growth performance of *M. laevigata* is presented in Table 2. Different growth traits showed variation among the accessions. Number of shoot varies from 10.17 – 23.33 followed by length of the longest shoot (80.83 – 205.33cm), inter nodal distance (4.56 – 9.15cm), 100 leaf weight (381.73 – 1093.02g), total shoot length (572.67-3277.83cm), moisture content (68.97 – 73.30%), moisture retention (61.11-83.62%) and leaf yield per plant (0.39 –3.00kg). Analysis of variance showed high significant variation at 1% level between accessions. The seasonal variation, interaction between accessions x season was significant at 1% level. The mulberry accessions reacts with the environment and yield component fluctuates due to environmental effect.

The simple and genotypic correlation coefficient showed that for all the parameter pairs, genotypic and simple correlation were in the same direction and the genotypic correlation were higher than the simple correlation indicating an inherent association between the parameters (Table 3). Number of shoot had high positive significant correlation with total shoot length (0.83**) and leaf yield (0.51*). Length of the longest shoot length was associated with total shoot length (0.72**) and leaf yield (0.67*), Inter nodal distance is associated with 100leaf weight (0.77**) and moisture retention capacity (0.68**). Hundred-leaf weight was associated with moisture retention (0.63*). Total shoot length is associated with leaf yield (0.67**). Likewise, leaf yield is associated with number of shoot (0.51*), length of longest shoot (0.67**) and total shoot length (0.67**). Similar results observed in case of genotypic correlation but the magnitude was higher than simple correlation. Such reports are available and reported by various authors (Bari *et al*., 1989; Tikader and Roy, 1999; Sarkar *et al*., 1987; Vijayan *et. al.,* 1997)).

The growth traits showed high biomass production and smooth leaf of some accessions (MI-0364, 0365, 0366). The accessions are suitable for silkworm rearing. The moisture retention capacity of the leaf is higher side i.e., more than 80%. The correlation of different growth traits indicates the suitability for selection of accessions. *M. Laevigata* harbors a number of agronomical important traits such as bigger leaf size, higher leaf thickness, moisture retention, resistance to biotic and abiotic stress like drought, saline and frost (Ravindran *et al*., 1999). Due to above positive characters, *M.laevigata* showed scope for introgression breeding between wild and cultivated species (Tikader and Dandin, 2005;Vijayan *et. al.,* 2004). Moreover, the breeding performance of *M. laevigata* showed positive results (Tikader and Dandin, 2007). So, the present results of evaluation of *M. laevigata* provide scope for selection and utilization in crop improvement programme.

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Table 1: Distribution of different mulberry accession used for the study.

State	Accession no.	Accession name	Mulberry species
Andaman islands	MI-0364	Lamia bay	*M. laevigata*
	MI-0365	Doomarnali	*M. laevigata*
Arunachal Pradesh	MI-0418	Chessa lobed	*M. laevigata*
Assam	MI-0343	Penagree	*M. laevigata*
Madhya Pradesh	MI-0315	Dhar	*M. laevigata*
	MI-0340	Badodhi	*M. laevigata*
	MI-0363	Bilaspur-2	*M. laevigata*
Manipur	MI-0427	Moulvaiphei	*M. laevigata*
Meghalaya	MI-0353	Sung Valley	*M. laevigata*
Sikkim	MI-0429	Sipsu	*M. laevigata*
Tamil Nadu	MI-0383	Yercaud lobed	*M. laevigata*
Uttaranchal	MI-0366	Resham Majri - 1	*M. laevigata*
	MI-0371	Resham Majri - 8	*M. laevigata*
	MI-0531	Haridwar - 2	*M. laevigata*
West Bengal	MI-0380	Saravathi	*M. laevigata*

Table 2. Mean performance and variability in growth traits of M. laevigata.

Acc.	NSH (no)	LLS (cm)	INTD (cm)	HUN_ lwt (g)	TSL (cm)	MC %	MRC %	LYP (kg)
MI0315	23.33	129.50	6.12	787.18	2009.00	69.16	70.27	2.98
MI0340	25.67	156.17	4.98	669.74	3331.50	69.27	67.84	1.36
MI0343	17.50	159.00	7.31	971.35	2070.83	71.00	70.95	2.29
MI0353	16.50	158.17	7.70	912.57	1852.33	69.98	77.34	1.66
MI0363	20.17	134.67	6.02	646.35	1907.17	71.36	76.94	1.57
MI0364	12.50	146.00	9.15	907.34	1198.50	70.71	83.62	1.37
MI0365	14.17	105.83	7.08	788.16	999.67	68.98	82.32	1.18
MI0366	28.00	201.50	6.89	621.80	3277.83	68.97	68.69	2.17
MI0371	20.67	169.00	5.88	371.07	2352.17	69.22	64.89	2.02
MI0380	15.33	131.83	7.33	810.93	1321.50	72.63	77.34	1.37
MI0383	11.50	205.33	8.68	1093.02	1958.17	72.95	73.19	2.35
MI0418	18.83	203.83	6.69	583.08	3260.00	70.18	67.45	3.00
MI0427	10.17	149.00	7.63	926.47	1128.50	69.72	71.54	1.27
MI0429	11.17	80.83	4.56	381.75	572.67	73.30	61.11	0.39
MI0531	24.17	158.17	7.26	614.17	3144.00	72.08	69.78	2.67
F-Value								
Acc.	**	**	**	**	**	**	**	**
Season	**	**	**	**	**	**	**	**
Acc. x Season	4.51**	9.04**	38.23**	369.74**	10.16**	40.00**	113.70**	189.25**

**, Significant at 1% level
NSH = Number of shoot, LLS = Length of the longest shoot,
INTD = Inter nodal distance, HUN_wt.= 100 Leaf weight, TSL = Total shoot length,
MC = Moisture content, MRC = Moisture retention capacity, LYP = Leaf yield /plant.

Table 3. Correlation coefficient in growth parameters of M. laevigata.

Parameters	X1	X2	X3	X4	X5	X6	X7	X8
X1	---	0.37	-0.43	-0.41	0.87**	-0.59*	-0.35	0.54*
X2	0.33	- - -	0.42	0.23	0.72**	-0.30	-0.19	0.68**
X3	-0.39	0.39	- - -	0.84**	-0.16	0.09	0.79**	0.25
X4	-0.40	0.21	0.77**	---	-0.24	0.15	0.69**	0.13
X5	0.83**	0.72**	-0.16	-0.23	---	-0.45	-0.45	0.67**
X6	-0.45	-0.18	0.11	0.07	-0.32	---	0.11	-0.48
X7	-0.31	-0.13	0.68**	0.63*	-0.37	-0.07	---	-0.24
X8	0.51*	0.67**	0.21	0.11	0.67**	-0.20	-0.16	---

*, **, Significant at 5% and 1% level, Upper diagonal genotypic correlation and Lower diagonal simple correlation
X1 = Number of shoot, X2 = Length of the longest shoot, X3 = Inter nodal distance,
X4 = Hundred leaf weight, X5 = Total shoot length, X6 = Moisture content,
X7 = Moisture retention capacity, X8 = Leaf yield / plant.

B. BOMBY XMORI SECTION

Heterosis Expression in Some Main Quantitative Breeding Characters in Four – Way Sex-Limited for Larval Markings Silkworm, Bomby mori L. F_1 Hybrids.

P. I. Tzenov, Sericulture Experiment Station, 24 Mito Orozov Str., Vratza 3000, Bulgaria.

ABSTRACT

Four Bulgarian sex-limited for larval markings silkworm pure breeds (2 of Japanese and 2 of Chinese type), 2 simple hybrids between them of the type J x J and C x C and 2 four – way F_1 hybrids have been used in this study. During the period 2003 – 2007 all the breeds and hybrids have been reared simultaneously during the spring (May) season each in volume of 4 replicates consisted of 200 female and 200 male larvae, counted after the 3rd moult. The main breeding quantitative characters such as cocoon weight, shell weight, shell percentage, cocoon filament length and weight, raw silk percentage and the reelability were recorded in each breed and hybrid. The heterosis expression in F_1 (the four way hybrids) with respect to the 4 pure breeds as well as the parental simple hybrids was calculated both for the mid-parent value (MP) and the higher parent value (HP).

It's estimated that the heterosis expression obtained in the four-way hybrids, calculated as regards the four pure breeds was comparatively high and positive. If compare the four-way hybrids with the parental simple hybrids however in most of the traits studied a low positive or even a negative heterosis expression was detected. It's brought out that considering the comparatively high positive heterosis expression detected in both the simple parental hybrids and in the four-way hybrids as well, compared with the pure breeds, the sex-limited for larval markings breeds studied should be used for commercial hybridization as four – way hybrids in order to obtain higher cocoon and egg yields.

Key words: silkworm, Bombyx mori L., four-way, hybrids, sex-limited, heterosis

1. Introduction

The silkworm breeds may form simple (A x B), triple [(A x B)x C] and double (four-way) crosses [(A x B) x (C x B)]. It is considered that the simple cross hybrids display a stronger hybrid vigor. On studying different types of crosses, it was found that double and triple hybrids do not show bigger variation on account of the commercial traits of cocoons compared with simple ones. For triple hybrids it is difficult to choose between hybrids of the [(Japanese x Japanese) x Chinese] type and these of the [(Chinese x Chinese) x Japanese] type, though the quality of silk from the latter is considered better (Lea, 1993, 1996).

Double crosses of the [(Japanese x Chinese) x (Japanese x Chinese)] type produce cocoons not enough uniform in shape and in thread length compared with those from the [(Japanese x Japanese) x (Chinese x Chinese)] type, which is preferable. There are no significant differences between the simple, three and

four-way hybrids, except for the fecundity characters in the parents which are higher in three and four-way crosses, compared with the simple hybrids.

In all the three types of commercial hybrids it was detected a comparatively high heterosis expression, both for the mid-parent value (MP) and the higher parent value (HP) as regards the main quantitative traits, such as cocoon weight, shell weight, shell percentage as well as the complex character fresh cocoon yield by one box of silkworm eggs. (Lea, 1993, 1996; Nacheva, et. al.2000)

However in crossing of silkworm breeds, having very big differences between their productivity the main quantitative characters inheritance in F_1 is intermediate with a high and positive heterosis expression for the MP and negative heterosis for the HP (Tzenov et al., 1999).

In fact the production of four – way F_1 silkworm hybrids is not the only way to make more effective the egg production. As it's a well known that the sex discrimination is one of the most laborious jobs in the hybrid silkworm egg production. In order to make easier and more precise this process sex-limited silkworm breeds have been created (Tazima et al., 1951; Strunnikov and Gulamova, 1958; Mano et al., 1969, Kimura et al, 1971)

The present study aimed at investigate the inheritance of some major quantitative breeding characters in some Bulgarian four – way F_1 silkworm hybrids between sex-limited for larval marking breeds.

2. Material and methods

The following Bulgarian silkworm breeds and hybrids, created by P. Tzenov were used in the study:

SN1, Iva1, Magi 2, Nova 2, SN1 x Iva1, Magi2 x Nova2, SN1 x Iva1 x Magi2 x Nova2, Magi2 x Nova2 x SN1 x Iva1. The pure breeds and the parental hybrids have the following main characteristics:

SN1: Uni-bivoltine Japanese type line. The egg serosa color is light gray, chorion color is white and the eggs are sticky. Sex-limited for larval marking – the females are zebra, the males are with normal marking. The cocoons are white, elongated with constriction.

Iva 1: Bivoltine Japanese type line. The egg serosa color is brownish, chorion color is yellow and the eggs are sticky. Sex-limited for larval marking – the females are with normal markings, the males are plain. The cocoons are white, dumbbell with high constriction.

Magi 2: Uni-bivoltine Chinese type line. The egg serosa color is green - grayish, the chorion color is yellow, the eggs are sticky. Sex-limited for larval marking – the females are zebra, the males are plain. The cocoons are white in color, oval.

Nova 2: Uni-bivoltine Chinese type line. The egg serosa color is green - grayish, the chorion color is yellow, the eggs are sticky. Sex-limited for larval marking – the females are with normal markings, the males are plain. The cocoons are white in color, oval.

SN1 x Iva 1: Bivoltine Japanese type simple hybrid. The egg serosa color is gray, chorion color is white and the eggs are sticky. The last instar silkworm larvae are bluish-white in color, the female individuals have zebra marking and the male ones are with normal markings. The body shape is thinner and longer. The cocoons are white in color, elongated with high constriction.

Magi 2 x Nova 2: Uni-bivoltine Chinese type simple hybrid. The egg serosa color is gray, chorion color is white, and the eggs are sticky. The last instar silkworm larvae are bluish-white in color, the females are zebra marked and the males are plain. The body shape is thicker and shorter. The cocoons are white in color, with oval shape.

(SN 1 x Iva 1) x (Magi 2 x Nova 2) and the reciprocal cross. The egg hatchability is 98 – 99 %, the pupation rate is 96 – 98 % and the larval duration is 26 –28 days. The cocoons are white in color, elongated, big with a mean weight 1.90 – 2.20 g. The cocoon shell weight is 0.420 – 0.490 g, the cocoon shell percentage is 22 %, the filament length is 1200 – 1300 m, the reelability is over 90 % and the raw silk percentage is 42 – 44 %. The fresh cocoon yield by one box of silkworm eggs is 36 – 39 kg. The hybrid is sex limited for larval markings. The females are with zebra marking and the males are with normal markings and plain.

During the period 2003 – 2007 all the breeds and hybrids were reared simultaneously during the spring (May) season each in volume of 4 replicates consisted of 200 female and 200 male larvae, counted after the 3rd moult. The main breeding quantitative characters such as cocoon weight, shell weight, shell percentage, cocoon filament length and weight, raw silk percentage and the reelability were recorded in each breed and hybrid. The heterosis expression in F_1 (the four way hybrids) with respect to the 4 pure breeds as well as the parental single hybrids was calculated both for the mid-parent value (MP) and the higher parent value (HP) using the following formula:

$$Heterosis = \frac{\overline{X_{F1}} - \overline{X_{MP}}}{\overline{X_{MP}}} \times 100 \quad (\%)$$

$$Heterobeltiosis = \frac{\overline{X_{F1}} - \overline{X_{HP}}}{\overline{X_{HP}}} \times 100 \quad (\%)$$

Where
HP = High parent
MP = Mid parent

3. Results and discussion

The results obtained are presented in Table 1. It is evident that as regards the traits cocoon weight and cocoon shell weight the four-way hybrids manifested a positive heterosis expression for the MP and negative heterosis for the HP if compared with the pure breeds and negative heterosis as regards the parental hybrids.

The results show that these two characters values in the four-way hybrids are a little bit lower and not significantly different compared with their parental simple hybrids, therefore the heterosis expression obtained in the simple hybrids is preserved in some extent in the four-way crosses but it has not been increased.

The data obtained show that in the shell ratio, filament length and weight, raw silk percentage and reelability, with some exceptions is manifested a positive heterosis expression as for the MP and negative heterosis as for the HP.

As a whole we may conclude that if compare the characters values in the four-way hybrid with those in the initial pure breeds, (but not with the parental single hybrids of the type J x J and C x C) the heterosis expression in the four way hybrid is also comparatively high. However if compared with the parental simple hybrids in most of the characters studied it is estimated low or even negative heterosis expression in the four-way hybrids.
Other conclusion which can be made is that when high heterosis expression has been obtained in the single hybrid, it can not be expected so high heterosis expression in the four-way hybrid as for the single hybrid as well, but the heterosis expression expected may be low positive only.

4. Conclusion

Considering the comparatively high positive heterosis expression detected in both the simple parental hybrids and in the four-way hybrids as well compared with the pure breeds the sex-limited for larval markings breeds studied should be used for commercial hybridization as four – way hybrids in order to obtain higher cocoon and egg yields.

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Table 1. Heterosis expressions in F_1 in four-way hybrids between sex-limited for larval markings silkworm breeds.

Hybrid, heterosis expression	Characters, heterosis expression in %						
	Fresh cocoon weight (mg)	Cocoon shell weight (mg)	Shell percentage (%)	Cocoon filament length (m)	Cocoon filament weight (mg)	Raw silk percentage (%)	Reelability (%)
SN1 x Iva1 x Magi2 x Nova2							
As for the pure breeds							
MP	+ 7,93	+ 8,48	+ 0,13	+ 3,51	+ 11,84	+ 4,58	+ 0,65
HP	+ 2,30	- 1,13	- 5,50	- 10,21	+ 3,53	- 0,005	- 1,91
As for the parental simple hybrids							
MP	- 4,57	- 4,25	+ 0,88	+ 5,06	+ 4,29	+ 6,88	- 0,34
HP	- 8,55	- 5,61	- 1,62	- 2,59	- 1,75	+ 3,50	- 1,85
Magi2 x Nova2 x SN1 x Iva1							
As for the pure breeds							
MP	+ 6,73	+ 6,05	- 0,29	+ 1,05	+ 9,59	+ 1,33	+ 3,56
HP	+ 1,18	- 4,07	- 6,27	- 12,45	- 7,13	- 0,68	- 1,49
As for the parental simple hybrids							
MP	- 5,28	- 6,70	+ 0,05	+ 2,79	- 2,62	+ 5,76	+ 2,25
HP	- 9,60	- 7,99	- 1,10	- 5,68	- 3,57	+ 1,50	+ 1,94

Evaluation of Genetic Potential of Pure Lines of Silkworm for Breeding and Silk Production.

Shakil Ahmad Khan, Mubashar Hussain, Ghulam Sabir, M. Mehboob Ur Rehman and M. Sohail Anwar Ch., Sericulture Research Laboratory Lahore, Pakistan.

ABSTRACT

The fundamental aim of silkworm breeding is to get robust and sturdy silkworm larvae for easy rearing and production of best cocoons in quality and quantity for a high yielding cocoon crop and high grade of raw silk. In Pakistan, efforts are being made continuously to give boost to the sericulture industry by developing pure parental silkworm lines suitable for rearing for commercial purposes. The study was carried out to evaluate genetic potential of the eleven pure silkworm lines (PAK-1, PAK-2, PAK-3, PAK-4, M-101, M-103, M-104, M-107, S-1, PFI-1 and PFI-2) for breeding and cocoon production during 2006-2008 at Sericulture Research Laboratory, Lahore. The experiment was carried out in completely randomized design. on the basis of the index values ranking M-101 (57.55), PAK-3 (52.22), PFI-2 (52.04), PFI-1, (51.14) PAK-4 (50.52) were identified as good performer for various economically important traits and were selected for field trials, hybridization and other breeding programmes.

INTRODUCTION

Silkworm is not only a commercially important insect, it is also found to be an important laboratory tool. It is estimated that more than 3 000 silkworm strains are available all over the world due to various ongoing breeding programmes (Nagaraju, 2002; Thangavelu *et al*., 2003). The maintenance of pure silkworm genetic resources has become very important for meeting the desired objectives of the breeder for immediate or long-term utilization in silkworm seed production. For race improvement a good stock of parental races or lines is must because the principal objective of silkworm breeding is the improvement of the gene pool in various characters useful in practical sericulture. It is necessary to maintain the genetic resources in their original form for their rational use in different breeding and other research purposes (Mukarjee et al., 1999; Basavaraja *et al*., 2003; Thangavelu et al., 2003; Yamaguchi, 2003). In addition to maintenance, systematic study of resource material is also very important, not only for classification and characterization of varieties but also for the selection of promising parents to initiate various breeding programmes to ensure silk seed production on sustainable basis. Evaluation of genetic material also helps in identification of lines with special characters like longer filament length, fine denier, stress resistance, disease resistance, etc. (Li et al., 2001). Availability of diverse genetic stock, gives ample choice for the breeder in selection of initial parents of his desire. Even half of a good silkworm egg layings from a good genetic stock can potentially transform the sericulture scenario to a greater extent (Chandrashekaraiah and Ramesh Babu, 2003). Most of the damage to sericulture can be attributed directly to silkworm diseases, unfavourable weather conditions and poor harvest of mulberry leaves. Therefore, prevention of silkworm diseases and breeding of a silkworm variety with high productivity are important commercial aspects of sericulture.

Silkworm pure lines used in this experiment were the out come of inbreeding of

parent silkworm varieties of indigenous and exotic origin at sericulture research laboratories and research centers in Pakistan. Therefore, maintenance of resource material and their effective utilization has become very important. Most of the quantitative traits of commercial importance in silkworm are under complicated polygenic control under the influence of the environment. For synthesizing the potential polyvoltine cross breeds, usually, the high yielding polygeneic traits of bivoltine varieties and fitness traits of polygeneic nature are hybridized as proper selection of potential and homozygous parents is very important. Effective utilization of selected germplasm also plays an important role in saving the time of the breeder in the synthesis of new hybrids. In the present study, an attempt was made to evaluate and characterize inbred lines for cocoon production on the basis of evaluation index method, frequently used for evaluating breeds/hybrids (Rao *et al,* 2006). Germplasm evaluation was conducted to ascertain the genetic potential of various pure lines of silkworm for commercial exploitation. Since sericulture is practiced in diverse agro-climatic zones, systematic evaluation is needed for the proper utilization of the available lines. The information generated would be useful for future breeding programmes and commercial rearing.

MATERIALS AND METHODS

The selected lines (PAK-1, PAK-2, PAK-3, PAK-4, M-101, M-103, M-104, M-107, S-1, PFI-1 and PFI-2) were reared under standard rearing conditions (Krishnaswami, 1975; 1983) for six generations in spring and autumn seasons during 2006-2008. The young larvae (1^{st}~3^{rd} instars) were reared at 27~28 °C with 85%~90% relative humidity and the late age larvae (4^{th} and 5^{th} instars) were maintained at 24~26 °C with a relative humidity of 70%~80%. Each breed was maintained in three replications. At the beginning of 4th instar, 300 larvae were counted from each line and retained for further studies. Rearing was carried out under hygienic conditions. At the end of 5th instar, the spinning larvae were collected manually and mounted in plastic collapsible mountages. During the rearing period, larvae and cocoons were assessed for the yield parameters like fecundity, cocoon yield, pupation rate, cocoon weight, shell weight, shell ratio, filament length, raw silk (%), reelability (%) and neatness using the following formulae:

Cocoon yield/10 000 larvae=(weight of cocoons obtained (kg))X10000/(larvae retained after 3rd moult (300));

Pupation rate (%)=(No. of good cocoons+(No. of double cocoonsX2))X100/(larvae retained after 3 rd moult–uzi infestated cocoons);

Cocoon weight (g)=(wt. of 25 male (g)+25 female cocoons (g))/50;

Cocoon shell weight (g)=(wt. of 25 male (g)+25 female cocoon shells (g))/50;

Cocoon shell ratio (SR, %)=(single cocoon shell weight (g))X100/(single cocoon weight (g));

Silk filament length (m) =revolutions of aprovetteXcircumference of wheel (m). These data were analyzed further by using the evaluation index method. Based on these values obtained, ranks were given for each breed accordingly.

Evaluation index method

Evaluation index value (EI) for silkworm breed performance was calculated by using the following formula (Mano et al., 1993).

Evaluation Index (EI) =A-BχCX10+50

Where, *A* is mean (average) of the particular trait; *B* is overall mean ofparticular trait; *C* is standard deviation; 50 is constant.

RESULTS AND DISCUSSION

Among all the tested eleven silkworm lines the fecundity was recorded maximum in PFI-1(506) and minimum in M-101 (448) while the average fecundity was 480. Table 1

1.MEAN PERFORMANCE OF HHYBRID SILKWORM LINES

Silkworm lines	Fecundity (eggs per moth)	Pupation rate (%)	Yield (kg/10000 larvae)	Cocoon weight (gms)	Shell Weight (gms)	Cocoon shell Ratio(%)	Filament Length (m)	Hatchability (%)	Raw silk (%)	Mortality (%)
PAK-1	485	89.73	11.831	1.37	0.276	20.14	786	86	10.67	5.66
PAK-2	462	88.17	10.27	1.473	0.304	20.63	850	79	11.77	6.71
PAK-3	479	90.43	10.822	1.448	0.307	21.2	735	83	11.95	5.16
M-101	448	88.11	11.583	1.483	0.309	20.83	756	78	10.88	6.92
Pak-4	501	79.84	12.318	1.471	0.299	20.32	955	91	12.38	4.29
M-103	498	87.77	11.922	1.519	0.321	21.13	915	89	12.17	2.94
M-104	456	85.84	10.823	1.494	0.309	20.68	816	78	11.35	7.16
PFI-2	464	86.85	10.429	1.381	0.286	20.71	826	75	10.86	6.19
S-1	481	84.9	10.288	1.461	0.303	20.74	876	76	10.63	6.62
PFI-1	506	87.64	12.314	1.513	0.312	20.62	982	93	12.65	3.16
M-107	502	88.56	11.982	1.573	0.316	20.9	964	84	12.34	2.7
Mean	480.181	87.076	11.325	1.47145	0.303	20.718	860.09	82.909	11.60	5.228
Minimum	448	79.84	10.27	1.37	0.276	20.14	735	75	10.63	2.7
Maximum	506	90.43	12.318	1.573	0.321	21.2	982	93	12.65	7.16
Standard Deviation	20.32	2.86	0.726	0.041	0.0093	0.219	85.507	6.236	127.65	1.691

In all the lines pupation rate was noted above 80% except in PAK-4(79.84%) and maximum in PAK-3 (90.43%) while the average pupation rate was 87%.Single cocoon weight was found highest in M-107 (1.573gms) and lowest in PAK-1(1.37gms) with an average of 1.471gms.Maximum shell weight noted in M-103(0.321gms) and minimum in PAK-1(0.276gms) maximum shell ratio

was found in PAK-3(21.2%) and minimum in PAK-1(20.14%) showing an average of 20.71%. Highest cocoon yield was observed in M-101 12.31 kg and minimum in pak-2 10.27 kg while the average cocoon yield was found 11.32kg raw silk was maximal in PFI-1 (12.65%) while minimum in S-1(10.63%). Filament length was found maximum in M-107 (982m) and minimum was measured in PAK-3(735m) with mean value of 960 m.
Hatchability was also recorded during the rearing which was highest in M-107(90.43%) and lowest in PFI-2 (75%).The mortality of silkworm lines was recorded maximum in M-104 (7.16%) while minimum in M-107 (2.7%).(Table 1). On the basis of evaluation index method PAK-4(57.55), PAK-3(52.22), M-107 (52.04), PFI-1 (51.14) and PAK-4 (50.52) were identified as potentially better performers and were selected for further breeding programmes. (Table 2).

Table 2. EI Values for Different Traits of Polyvoltine Hybrid Silkworm Lines Studied.

Silkworm Lines	Fecundity (eggs per moth)	Pupation (%)	Yield (kg/10000 larvae)	Cocoon Weight (gms)	Shell Weight (gms)	Cocoon shell Ratio (%)	Filament Length (m)	Hatchability (%)	Raw Silk (%)	Mortality (%)	Mean Value
PAK-1	52.37	59.30	56.96	25.69	19.89	23.61	58	41.23	54.97	52.52	44.75
PAK-2	41.05	53.84	35.54	50.73	50.21	45.99	50	48.81	43.73	58.70	47.86
PAK-3	49.41	61.74	43.07	44.63	53.22	71.92	62.85	35.36	50.16	49.88	52.22
M-101	34.16	53.63	53.55	53.17	55.37	55.45	59.97	37.82	42.13	59.94	50.52
PAK-4	60.24	35.80	63.67	50.24	44.82	31.42	61.47	61.10	63	48.49	57.55
M-103	58.76	52.44	58.22	61.95	68.48	69.10	61.26	56.42	59.79	36.32	38.27
M-104	38.10	45.69	44.09	55.35	55.58	48.27	60.44	44.84	42.13	61.35	49.63
PFI-2	42.03	49.23	37.65	28.29	30.86	49.64	59.95	46.01	37.31	55.64	43.66
S-1	50.40	42.41	35.71	47.30	49.13	51	59.72	51.86	38.92	58.17	48.51
PFI-1	62.70	51.99	63.62	60.48	58.81	45.54	61.74	64.25	66.21	37.82	51.14
M-107	60.73	52.20	59.04	75.12	63.11	58.28	61.43	62.15	51.76	35.08	52.04

This study was an effort to ensure the effective utilization of the polyvoltine hybrid silkworm lines and the maintenance of the resource material. Keeping the importance of hybrids in view, the germplasm lines were reared consecutively for six generations and their quantitative traits were evaluated using reliable statistical method, i.e. evaluation index method to assess the performance of the inbred lines. Earlier many breeders (Mano *et al*., 1993; Gower, 1971; Ramesh Babu *et al*., 2001, Rao *et al*., 2004, Rao *et al*., 2006) analyzed their breeds by adopting the above methods either individually or together. The lines which have been selected through these methods could be effectively used in further breeding programmes as potential parents for synthesizing superior silkworm hybrids that are suitable for culture under sub-tropical climatic conditions.

Table 3.Ranking of Silkworm Lines on EI Value

S.NO.	SILKWORM LINES	EI VALUE	RANK
1.	PAK-1	44.75	9
2	PAK-2	47.86	8
3	PAK-3	52.22	2
4	M-101	50.52	5
5	PAK-4	57.55	1
6	M-103	38.27	11
7	M-104	49.63	6
8	PFI-2	43.66	10
9	S-1	48.51	7
10	PFI-1	51.14	4
11	M-107	52.04	3

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Investigation on improvement possibility of resistance, production and reproduction traits in 3P, 2P and P generations in three Japanese pure lines of silkworm *Bombyx mori* L., using individual selection in 3P generation.

A.R. Seidavi[1], S.Z. Mirhoseini[2], M. Mavvajpour[3], A.R. Bizhannia[3] and M. Ghanipoor[3] (1- Animal Science Department, Islamic Azad University, Rasht Branch, Iran, 2- Animal Science Department, Agriculture Faculty, Guilan University, Iran, 3- Iran Silkworm Research Center, Rasht, Iran).

Abstract

At silk cocoon production process and silkworm breeding programs, reproduction traits supplies silkworm egg producers, cocoon quantitative and resistance traits supplies farmers' benefits. Hence it must be noticed to these traits together. Furthermore, selection systems applied in 3P pure line levels. Reproduction, production and resistance characters have negative correlations probably in some varieties. Therefore, an experiment must be designed to investigation on effect of parent's selection on the basis of cocoon weight on reproduction and resistance characters in 3P, 2P and P generations. Purpose of this experiment was investigation on improvement possibility of resistance, production and reproduction traits in 3P, 2P and P generations in three Japanese pure lines of silkworm *Bombyx mori* L., using individual selection in 3P generation parent's level. At each pure line including 31, 103 and 107, it is recorded male and female cocoon weight and then 16 sire and dam parents were selected accordingly the most weight at each line. Furthermore 16 sire and dam parents were selected by chance and without any selection at each line. These three pure lines were reared in3P, 2P and P generations and investigated and compared their resistance, production and reproduction traits separately. From obtained results, it was showed that phenotypic trend for cocoon weight is positive and significant ($P<0.01$). It is showed that parental selection on the basis of single cocoon weight in 3P generation, had not decrease reproduction and resistance characters at next generations of 3P, 2P and P significantly ($P<0.01$). Hatchability, defected eggs percentage and pupae vitality were not declined significantly in three studied pure lines ($P<0.01$). These results could due non-negative correlations between these traits in three studied pure lines. From obtained results, parents would be selected on the basis of cocoon weight parameters in 3P generation. Also it is recommended that economical coefficients and genetical parameters are noticed for reproductive, resistance and quantitative cocoon characters together.

Keywords: Cocoon Weight, Parents, Line, Individual Selection, 3P generation, Silkworm

Introduction

Iran produced 2543 tons fresh cocoon and 395 tons raw silk annually at 2006 based on International Sericultural Commission statistics ISC (http://www.inserco.org/uk/ reglement.php?rub=2). Iranian farmers who reared

silkworm included 50000 families. Thus Sericultural industry has criteria and important role in rural development.

At silk cocoon production process, three traits groups are important included reproduction traits which supplies silkworm egg producers, cocoon quantitative and resistance traits which supplies farmers' benefits (Seidavi et al, 2004c). There are three separated level in silkworm breeding centers as 3P, 2P and P generations. Most of breeding programs conducted in 3P generation due their small population size.

Govindan et al (1991) and Seidavi et al (2004a) were reported cocoon weight and cocoon shell weight traits are under additive and dominance gene effects. Furthermore, it is reported additive genetic variance in more than dominance genetic variance for these traits. Hereditability of cocoon weight is between 0.03-0.49 and c Hereditability of cocoon shell weight is between 0.14-0.60 (Govindan et al, 1991; Malik et al, 1999, Seidavi et al, 2004b). Many researchers are emphasized on importance of correlation and hereditability estimations for silkworm economical traits in order to improvement and optimization of selection systems in silkworm egg production (Govindan et al 1991; Seidavi et al, 2004b).

At the present time, it is noticed to some traits e.g. cocoon weight, cocoon shell weight, pupae vitality, and hatchability percentage in total pure line systems. Parental generations are selected based on these traits. There are inconsistent reports from positive phenotypic correlations to negative phenotypic correlations between resistance, production and reproduction traits in different silkworm breeds (Datta et al, 2001, Seidavi et al, 2004a). Thus it must be investigated on effects of parental selection based on cocoon weight traits on resistance, production and reproduction traits in future generations i.e. 2P and P generations. These studies must conduct in different countries separately based on management and regional conditions of each country for total pure lines. From obtained result, producers can decide regarding selection system type in 3P pure line generation.

In fact, in Sericultural industry and silkworm breeding systems must emphasize on production, reproduction and resistance characters together and jointly. Because there is negative correlations between these production, reproduction and resistance characters in some commercial pure lines, hence individual selection of 3P parents based on cocoon weight traits must noticed based on their results in future generations i.e. 3P, 2P, P and hybrids. Purpose of this experiment was investigation on improvement possibility of resistance, production and reproduction traits in 3P, 2P and P generations in three Japanese pure lines of silkworm *Bombyx mori* L., including 31, 103 and 107 using individual selection in 3P generation.

Materials and Methods

It was constructed an original population included three Japanese of 3P pure lines of 31, 103 and 107 at first year of experiment. Cocoon weight, cocoon shell weight and cocoon shell percentage individually were recorded in male and female separately. Then per each pure line, two groups were constructed included selected and random groups. For this purpose in each pure line, 16 male and female individuals organized as selected group who had the highest

cocoon weight amongst original population. These male and female individuals were mated. Then in each pure line, 16 male and female individuals organized as random group who had the moderate and average cocoon weight in compare with original population. These male and female individuals were mated also. Thereafter silkworm eggs were produced from two groups as inbreeding mating in each pure line separately. Their offspring were conserved under standard protocols for one year (ESCAP, 1993). In second year, 12 families were hatched and reared for each group and pure line. These offspring were reared under similar conditions. Total characters including resistance, production and reproduction traits were recorded and analyzed. Obtained moths were mated randomly in each group and pure line separately for 2P silkworm egg production. In third year of experiment, 12 families were hatched and reared for each group and pure line. These offspring were reared under similar conditions. Total characters including resistance, production and reproduction traits were recorded and analyzed. Obtained moths were mated randomly in each group and pure line separately for P silkworm egg production. After silkworm egg hatching and rearing of P generation, total characters include resistance, production and reproduction traits were recorded and analyzed.

It was applied favorite conditions for moth emergence such as 25 centigrade and 75% relative humidity. Pure lines were reared under standards protocols in all four years. It was used rice straw as mabshi for cocoon spinning in each replication (family) separately. After cocoon spinning development (seven days after starting of cocoon spinning), obtained cocoons gathered and sorted based on form, thickness, clarity etc to four classes include good, middle, double and low cocoons. It was calculated ratio of each class cocoon for each replication separately. Furthermore, it was investigated on health or disease of total obtained pupae and calculated ratio of each class cocoon disease for each replication separately. It was recorded cocoon weight for good and double cocoons. All records were conducted on 8^{th} days of cocoon spinning. It was used for data analyzing from CRD model, GLM approach, and SAS software. Under model was used for data analyzing for each pure lines separately: $y_{ij}=\mu + G_i + e_{ij}$ which y_{ij} was record or observation from trait, μ was trait average, G_i was group effect (selected and random) and was e_{ij} residual effects. Furthermore, it was used appropriate transformation like angle transformation for those data which did not followed by normal distribution. DNMRT method was used for average compares.

Results and Discussion

Table 1 present summary of obtained results during four successive generations. As expected, direct selection in all three pure lines resulted to cocoon weight improvement in three successive generations (3P, 2P and P generations). Other studies confirm these results previously. Hereditably of cocoon weight were reported between 0.03-0.49 (Singh et al, 1998; Jayswal et al, 2000; Seidavi et al, 2004b). Response to selection is followed by selection intensity, trait hereditably and phenotypic deviations.

From table 1 is showed parental selection in 3P generation have not negative effects on Reproduction, production and resistance characters in studied Japanese pure lines ($P<0.05$). Previously, Jayswal et al (2000) reported similar

results. These results can be for positive correlations between reproduction, production and resistance traits. In all pure lines, cocoon selection in 3P generation did not result to any significant decrease for hatchability percentage, unfertilized eggs percentage and pupae vitality (Table 1).

The previous studies show that the variance of GCA in resistance characteristics (which represents additive genetical variance) is much higher in Japanese lines compared to Chinese lines (Mirhosseini et al, 2004). As a result in Chinese lines the non-additive genetical variance has a main role in diversity of resistance characteristics, while in Japanese lines the additive genetical variance for the number of survived larvae and pupae and the percentage of pupal survival were some times higher. The part of additive and non-additive genetical variance from the total variance of cocoon weight in Japanese lines was almost equal but in Chinese lines the cocoon weight was more affected by non-additive genetical effects (Mirhosseini et al, 2004). Also due to the results of research the cocoon shell weight and the percentage of cocoon shell weight are very much affected with non-additive genetical effects (Mirhosseini et al, 2004).

Individual selection correlated to vitality potential and gene flow from one generation to future generation. Natural selection in successive generations deleted and eliminated susceptible individuals. Hence, population becomes uniform and invariable for related alleles. Thus unflavored alleles eliminated from population (Mano, 1994; Bhargava et al, 1995).

Resistance is a quantitative trait with incessant distribution and affected by major genes and minor genes. Narasimaraju et al (1992) and Chen et al (1996) were reported silkworm resistance controlled by double dominance gene on un-sexual chromosomes. If there is random mates in successive generations of silkworm population, natural selection resulted to major genes and modifier genes.

Li (1992) suggested selection for each pure line is conducted separately. He recommended selection intensity were not same for all pure lines. At this research we understood obtained results were not similar in all three studies pure lines for hatchability percentage, unfertilized eggs percentage, pupae vitality, and other resistance, production and reproduction traits. It is due to the different heritabilities and phenotypic deviations in different pure lines. Therefore it is necessary estimating of genetics parameters for silkworm breeding systems (Nagaraja et al, 1996; Devaiah and Reddy, 1999).

In most of the varieties, the percentage of heterosis in the productive characteristics is higher than the percentage of heterosis for the survival of the larvae and the pupae. Between the cocoon characteristics, cocoon shell weight and cocoon shell percentage have the highest and the lowest amount of heterosis, respectively. This represents the high portion of non-additive effects in genetical control of this characteristic. High percentage of heterosis in productive characteristics could be illuminated with respect to the additive and non-additive genetical variance of cocoon characteristics (Mirhosseini et al, 2004).

From obtained results, parents could be selected on the basis of cocoon weight parameters in 3P generation. Also it is recommended that economical

coefficients and genetical parameters are noticed for reproductive, resistance and quantitative cocoon characters together.

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Table 1. Effect of individual selection on resistance, production and reproduction traits in three Japanese pure lines in successive generations[a].

	Pure Line→	31		103		107	
	Traits↓ Selection System↓	Individual Selection	Random Selection	Individual Selection	Random Selection	Individual Selection	Random Selection
Resistance Traits	Alive Larvae Number (no)	273.42[a]	236.46[b]	269.6	378.7	6.96163333	244.71
	Alive Pupae Number (no)	237.29[a]	207.71[b]	343.9	217.9	232.00	229.54
	Pupae Vitality Percentage (%)	86.538	85.841	82.11	80.38	89.774	93.670
	Pupae Vitality Percentage in Best Cocoon (%)	91.877	90.686	87.693	89.959	94.414	97.354
	Pupae Vitality Percentage in Middle Cocoon (%)	80.224	78.723	68.798	73.488	85.636	90.485
Production Traits	Produced Cocoon Number (no)	264.42[a]	231.29[b]	263.43	252.17	244.63	235.33
	Best Cocoon Number (no)	202.58[a]	177.71[b]	195.26	196.63	172.83	172.67
	Middle Cocoon Number (no)	45.625	41.042	48.826[a]	35.667[b]	53.250	45.417
	Low Cocoon Number (no)	7.083	6.917	12.913	9.792	7.125	4.917
	Double Cocoon Number (no)	9.1250[a]	5.6250[b]	6.435[b]	10.083[a]	11.417	12.333
	Best Cocoon Percentage (%)	76.306	75.722	74.928	77.880	70.394	72.886
	Middle Cocoon Percentage (%)	17.492	17.754	17.782	14.476	21.550	19.574
	Low Cocoon Percentage (%)	2.771	4.065	4.8491	4.0317	3.428	2.151
	Double Cocoon Percentage (%)	3.4325[a]	2.4592[b]	2.4400[b]	3.6108[a]	4.6275	5.3892
	Best Cocoon Weight (gr)	330.07[a]	280.63[b]	322.33	311.85	252.43	241.53
	Double Cocoon weight (gr)	3.20522	3.16400	3.1213	3.2327	2.9317	3.0971
	Single Best Cocoon Weight (gr)	1.65546	1.59783	1.65900	1.63038	1.47296[a]	1.42154[b]
	10000 Larvae Cocoon Weight (gr)	17056.1	16455.1	17043.8	16930.4	15669.6[a]	15096.6[b]
	Larval Duration (hr)	623.333	623.875	625.7174	625.2500	615.1667[b]	618.5000[a]
Reproduction Traits	Hatched Larvae (no)	485.50	477.13	560.96[a]	506.13[b]	572.92	546.67
	Un-hatched Eggs (no)	64.96	67.33	26.826	26.000	14.292	17.625
	Unfertilized Eggs (no)	10.500	23.875	20.696	13.292	31.125	29.208
	Hatched Eggs Percentage (%)	86.127	83.824	92.416	92.814	92.7192	92.0438
	Un-hatched Eggs Percentage (%)	11.995	12.000	4.1804	4.8113	2.2696[b]	2.9796[a]
	Unfertilized Eggs Percentage (%)	1.879	4.175	3.4009	2.3733	5.0121	4.9775
	Hatchability Percentage (%)	87.807	87.630	95.6730	95.0900	97.6196[a]	96.8596[b]
	Total Produced Eggs (no)	560.96	568.33	608.48[a]	545.42[b]	618.33	593.50

[a]**There is significant difference between the numbers that are shown with the different letter(s) in each row for each pure line. Each group of data without any letter has got no significant differences.**

Studies on establishment of silkworm parthenogenetic clones and its application.

Wang Yong-qiang [1], He Ke-rong[1], Zhu Xing-rong[1], Liu Xin-Ju[1], He Xiu-ling[1],Yao Yao-tao [2]

1.Sericultural Research Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

2. Sericultural Research Institute, Huzhou Academy of Agricultural Sciences, Huzhou Zhejiang 313000, China.

Abstract

Based on genetic characters of all female generations and identical genotypes, the silkworm parthenogenetic clones have an important value on the silkworm breeding and genetic mechanism analysis of heterosis. In this paper, the progress during recent 10 years was introduced, including the study of genetic characters, establishment of practical parthenogenetic clones and its application on silkworm breeding.

Keywords : *Bombyxmori* ; Silkworm parthenogenetic clones ; Establishment ;

Application; Progress

Silkworm (*Bombyx mori)* is an important economic insect, male and female individuals have different economic characters and value. The technology of"Rearing only male silkworm in cocoon production"could improve comprehensive economic efficiency of sericulture and silk industry. On the other hand, the technology of "Rearing more female silkworm in eggs-producing station"could reduce the cost of egg production. It is the sericultural researcher's pursuit of realizing above goals by the technology of sex control. Up to now, the technology of rearing male silkworm has been practically used in cocoon production. Commercial varieties of male silkworm have been bred and popularized China, such as "Qiuhua XPing30", "Qiufeng XPing28"[1]. Establishment of the practical silkworm parthenogenetic clones is an effective mean to achieve the target of"Rearing more female silkworm in egg-producing station". Parthenogenesis (also known as asexual reproduction, parthenogenetic clone) refers to the phenomenon of female reproductive cells developed directly to individual without fertilization. Silkworm parthenogenetic clone is the genetic group of all female generation and identical genotype through the selecting and breeding of parthenogenetic technology. A series of re searching works were carried out since 1996, which included the genetic mechanism of parthenogenetic characters, establishment of practical parthenogenetic clones and its application on breeding. The main research progresses are summarized in the present paper.

1 Genetic mechanism of parthenogenetic character

The rate of silkworm parthenogenesis in silkworm is very low in natural condition, but the artificial stimulation by physical and chemical factors can increase the rate. The most efficient method is the hot water treatment developed by B.L. Astaurov and V.A.Strunnikov in Russia[2]. According to the feature of bivoltine silkworm resources, the parthenogenetic conditions of hot water treatment were optimized, the appropriate temperature and time were decided as 44-46 ℃, 14-18 min respectively [3]. At the same time, the parthenogenetic rate of 16 silkworm pure lines and four pairs of hybrid

were investigated, the rate were between 34.20-86.76% (average of 51.13%) and 75.0-90.13% (average of 79.00%) respectively. There was a significant difference between varieties, and the parthenogenetic rate of hybrid was higher than pure lines. Therefore, it was deduced that the genetic factors was a major factor influencing the parthenogenetic rate [4-5].

Based on the multiple regression procedure for the generations mean value analysis, genetic effects had been analyzed on silkworm parthenogenetic character. The results were as follows: the hybrid vigor rate was 20.71%, the narrow heritability was 74.02 % and more than one pair of major genes controlled the heredity. The rates of parthenogenetic developed eggs varied markedly among different generations, the genetic effect of silkworm parthenogenetic character was subordinated to additive and dominant model, controlled by additive effect, then dominant effect. The additive and dominant effect was 97.05% of total genetic and variation [6-7].

2. Establishment of practical silkworm parthenogenetic clone

Based on analysis of the genetic characteristics of parthenogenesis, bivoltine varieties with good economic characters were generated continuously by parthenogenesis so as to improve hatching rate of parthenogenetic generations (Because female and male generations of conventional varieties was 50/50, the silkworm parthenogenetic clones have practical value on breeding if its hatching rate is over 50%). The breeding objectives of silkworm parthenogenetic as followed: parthenogenetic rate and hatching rate reached 90 percent and 80 percent, with fine cocoon and silk quality.

In the early generations of selection, the parthenogenetic rate of some varieties could reach 70 ~ 90%, however, the hatching rate is very low and only 1or 2 larvae hatched in some varieties. The deformity silkworm larvae were often observed and difficult to rear. After 9th generation, the hatching rate markedly improved and the larvae were reared easily gradually. After 10 years of breeding, the resource pool of bivoltin silkworm parthenogenetic clones has successfully established, including 25 clones, in which 11 Chinese parthenogenetic clones, 11 Japanese parthenogenetic clones and 3 hybrid parthenogenetic clones. The parthenogenetic rate and hatching rate of 13 parthenogenetic clones were above 90% and 80% respectively with stable economic characters and breeding value.

3. Application of silkworm parthenogenetic clones on breeding

3.1 The breeding of single-cross combination hybridized with parthenogenetic clone and sex-limited egg color variety

Using the practical parthenogenetic clones as female parent, the sex-limited egg color variety with fine economic characters as the male parent (female and male individuals could be separated by different egg color), a new hybridizing mode of silkworm breeding and egg production (called single cross variety) have been realized by the technology of sex control. Utilizing this model together with the multiple mating ability of male moth, the rearing ratio of female and male larvae could be increased from the current 1:1 to 2:1 or higher, which will realize the goal of “Rearing more female silkworm in egg-producing station”, decrease the rearing scale more than 25% and reduce the cost of silkworm egg production effectively. Certainly, new silkworm variety

with extraordinary economic characters would be expected to breed out by this model. In other words, different selecting priorities will be carried out by making full use of sex-linked genetic laws (For example, cocoon and silk quality as selecting key to female parent and viability as selecting key to male parent).

To test the feasibility applying single cross model on silkworm breeding and egg production, forty-two pairs of single-cross combinations by the method of incomplete diallel were reared under the same conditions (commercial silkworm variety "QiufengXBaiyu" as the control) and their economic characters and combining abilities were investigated. Based on initial screening and further comparison tests, several fine single cross combinations have been bred out, such as Female29Xegg36, Female35Xegg36 and etc. The major economic characters of these combinations were superior to control [8-9]. For example, results of laboratory identification to "Female 29 Xegg 36" in the autumn of 2007 indicates: larva-pupa rate was 93.76 percent, 0.32 percentage points higher than the control, cocoon and cocoon shell weight per 10000 silkworms were 16.27 kg, 3.736 kg respectively, 10.23% and 19.28% increased to compare with the control respectively. In addition, this single-cross combination was reared in farmer's condition in the autumn of 2007, cocoon yield per box of eggs was 51.5 kg, 8.42% higher than the control.

3.2 The breeding of male silkworm variety hybridized with parthenogenetic clone and balanced lethal male silkworm variety.

At present, the technology of "Rearing male silkworm in cocoon production " has been popularized in main sericulture regions of China, such as Zhejiang, Shandong, Yunnan, Guangxi and etc., which promotes the economic benefit of sericulture.

The new varieties of male silkworm have been gradually bred out in recent years, including"QiuhuaXPing30", "QiufengXPing28","Xian7XPing48",among of which Qiuhua, Qiufeng and Xian7 are sex-limited marking varieties, Ping30, Ping28 and Ping48 are varieties of balanced lethal male silkworm. During larval rearing, male individuals could be picked out by the different marking to decrease the cost of egg production. If the practical parthenogenetic silkworm clone is used instead of the sex-limited marking variety, the egg production cost of male silkworm variety could be decreased greatly.

In recent years, thirty pairs of male silkworm combinations were prepared by hybridized silkworm parthenogenetic clones with balanced lethal male silkworm varieties. Through comparative test for two years in laboratory, three pair of combinations (Female35XPing28, Female29XPing28, Female29XPing30) were selected with the performance of larva-pupa rate, cocoon shell weight per 10000 silkworms and raw silk rate of fresh cocoon being superior to the control (QiufengXBaiyu), excellent reelability and neatness. Compared with the male silkworm variety of"QiufengXPing28", the viability and silk quality were similar and the yield was superior to the control.

For example, the results of laboratory identification to "Female 35 XPing28" in the autumn of 2007 showed that larva-pupa rate was 91.82 percent, neatness 94.0 points, none-broken filam ent length 930m, raw silk rate of fresh cocoon 18.84 percent, which were similar to the control of"QiufengXPing28". Cocoon and cocoon

shell weight per 10000 silkworms were16.27 kg, 3.736 kg respectively, which were 4.5% and 8.56% higher than the control [10-12].

4. Outlook

Through the research work over 10 years, the genetic mechanism of parthenogenesis was clarified, the resource pool of practical silkworm parthenogenetic clones was established and parthenogenetic clones had been applied into the silkworm breeding. The model of single cross will not only innovate the patterns of silkworm genetic breeding and egg production, but increase the efficiency of hybrid production and decrease the cost of egg production as well. The hatching rate and economical characters of parthenogenetic clones will be further improved in future research. The single cross combination with fine economic characters will be tested by laboratory-joint-test and farmers' rearing, which include the combinations hybridized with practical parthenogenetic clones and the sex-limited egg color variety or balanced lethal male silkworm variety.

Moreover, the parthenogenetic clone is the good material for the study of heterosis mechanism, quantitative traits and epigenetic analysis because of its unique structure of the genotype. And related study will be carried out gradually in the future.

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Phenotypic characterization of Silkmoth Races from the Genetic Stock of Bombyx mori L. Sp. In Romania.

Alexandra Matei[1], Magda Androne[1], I. Pacsaj[2], Christina Bojan[2]. ([1]CS SERICAROM SA-Research Department, Bucharest, Romania, [2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania).

ABSTRACT

The importance of gene bank existence as an ess ential condition for breeding programs elaboration is unanimously known for every plant and animal species, from which the permanent preoccupation for its diversification and maintaining by appropriate proceedings of preservation “in situ” or “ex situ”. This way, there is being avoided the loss of biological material, especially of the local races resistant to diseases and adapted to environmental conditions. This study aims the analysis of phenotypic characters variability within the genetic stock of Bombyx mori sp., in accordance with its biological development stages (egg, larva and pupa). Native genetic stock of silkmoth Bombyx mori L. sp. resulted by: identification of local populations gene sources, bilateral exchange of biologic material with similar foreign institutes, creation of new genotypes using specific breeding methods. Within its structure, the genetical stock of silkmoth include 72 races.

The silkworm specific experimental technique has been applied, differentiated by technological and biological development stages. The sample size that were the base for phenotypic parameters determination as well as the working methods correspond to sericulture technical standards. The main phenotypical and quantitative parameters of the races that represent the gene stock of Bombyx mori sp., have the following values: number of eggs/laying (230-710 eggs/laying), hatchability (80.6-100%), larval stage duration (26-32 days), larvae weight (4.2-5.7 g), larvae pupation rate (80.8-96.6%), raw cocoon weight (1.445-2.361 g), cocoon shell weight (0.240-0.520 g), fiber length (746-1356 m), metric number of fiber (2917-3764 m/g). Depending on the quantitative parameters value, the silkworm races are being used differently, entire genetic stock being destined for various technological levels, as follows: 4 active races (parents of hybrids), 4 candidate races for parents of hybrids, 64 races in preservation.

Key words: Bombyx mori, genetic stock, raw cocoon weight, cocoon shell weight, fiber length

INTRODUCTION

The heredity, variability and selection represents the main factors of the animal and vegetable organisms evolution. If heredity provides the resemblance of the individuals from successive generations, the variability represent the inconsistent side of heredity, determining the differences between individuals that exists more or less to all the living beings groups. The presence of variability makes the application of selection possible, action that leads to improving animal populations.

The necessity of studying the conservation of genetic stock, has been taken into consideration by many authors (Hebean V., 1986; Brasla A. and coll., 1989;

Thangavelu K., 1997; Tzenov P., 2002; Petkov N., 2004; Matei A., 2004, 2007), existing two reasons for which the animal populations need to be preserved:
a) the statute of being in menace of disappearance;
b) their genetic value.
Taking into consideration the structure and functions of the organisms to whom the variability operates to, there are being dinstinguished: morphological variations including shape and size changes of the body regions or organs; physiological variations which refers to physiological processes, especially to the ones with implications upon some economical characters like production, food conversion, fecundity; structural variations regarding the structure of organs and tissues.
The variability of individuals which form a population, it refers both on quantitative and qualitative features, on this aspect being distinguished: quantitative variations that can be measured, which refers to differences between metric characters and mostly with economical implications; qualitative variations that can't be measured, they only can be described.

MATHERIALS AND METHODS

The biological material has been represented by 72 races consisting of the gene stock of Bombyx mori sp., grouped by their origin.
The silkworm specific experimental technique has been applied, differentiated by technological and biological development stages (Grekov D., 2005). The sample size that were the base for phenotypic parameters determination as well as the working methods correspond to sericulture technical standards.

RESULTS AND DISCUSSION

1. The variability of egg phenotypic characters

1.1 The variability of egg morphological characters

Egg size. The egg size at Bombyx mori L. sp. races is presented as follows: length 1.4 mm (native races), 1.3 mm (chinese races), 1.5 mm (japanese races) and 1.1 mm (tropical races), and the egg width varies between 0.89 and 1.02 mm.
The egg weight is 0.50 mg (native races), 0.49 mg (chinese races), 0.54 mg (japanese races) and 0.39 mg (tropical races).
Number of eggs/laying (Table 1). Concerning Bombyx mori sp., the number of eggs/laying ratio varies between 200 and 800.
This character is being influenced by race, food quality provided to larvae, temperature and humidity conditions during laying depose.
In case of the races existing within the genetic sericultural stock, the number of eggs/laying by race group registered values between 490 and 710 eggs/laying (native races), 276-562 eggs/laying (japanese races), 276-616 eggs/laying (chinese races) and 230-450 eggs/laying (tropical races).

Table 1
Egg biological parameters

Race groups	Number of eggs/laying		Hatchability (%)	
	Min	Max	Min	Max
Native races	490±10	710±12	90.0±0.47	99.0±0.47
Japanese races	276±11	562±6	81.3±1.70	99.6±0.47
Chinese races	276±2	616±15	80.6±1.89	100.0±0.21
Tropical races	230±16	450±29	83.6±2.49	97.6±1.25
Races average	318±9	584±15	83.9±1.64	99.1±0.60

Egg colour. During laying depose the egg colour is gradually yellow and in the next 3-4 days its colour becomes violet-pink and in the end the final colour is grey with different shades: dark grey, light grey, greenish grey but also orange, pink etc. All of these refers to embryonated egg colour.
The chorion colour, visible after larvae hatching, presents a serie of mutants: white, light yellow or dark yellow, green, grey. Being a race character, both the embryonated egg colour and chorion colour represent a silkworm selection character.
The races existing in sericultural native genetic stock present the egg colour in different shades: dark grey (japanese races), greenish-grey (chinese races), meanwhile the chorion is white at the first races group and yellow at the second one.
1.1. The variability of eggphysiological characters
The voltinism (generations/year) represent a physiological character determined by environmental and genetical factors. Between environmental factors, the temperature and light plays an essential part. Incubating eggs from bivoltine races at the temperature 15-18^{o}C and short photoperiod (less than 12 hours), silkmoth appear and depose non-hibernated eggs, that is 2 generations/year, in case of incubating eggs at high temperature (25-26^{o}C) for a longer photoperiod (more than 12-14 hours), silkmoth appear and depose hibernated eggs (one generation/year).
The genetical determinism of voltinism is being attributed to a number of 3 multiple sex alleles (Hs, Hs^2, h_S) modified by a number of autosomal genes (H1, h_1, H_2, h_2, H_3, h_3).
The structure of sericultural native genetic stock includes bivoltine races (tropical type).
The hatchability, by race group, varies between 90.0 and 99.0% (native races), 8.06-100.0% (chinese races), 81.3-99.6% (japanese races) and 83.6-97.6% (tropical races).
2. The variability of larva phenotypic characters
2.1. The variability of larva morphological characters
The larvae length is influenced by external factors, such as the rearing conditions, feeding but it also represents a race character specific to breeding races. By race group, the larvae average length is presented in Table 2.
Larvae weight is influenced by the factors that determined the previous character and their variability by race group is presented in Table 2.

Table 2
The variability of adult larvae length and weight by races groups

Race groups	Larvae length (cm) X±Sx	Larvae weight (g) X±Sx
Native races	7.6±0.02	5.7±0.12
Chinese races	6.2±0.06	5.1±0.10
Japanese races	7.0±0.03	5.6±0.08
Tropical races	5.8±0.06	4.2±0.11
Races average	6.7±0.04	5.2±0.10

Larvae colour represents a complex and variable character and refers to the tegument cephalic capsule and eyes.
In the breeding works, tegument colour and larval marks are selection criteria taken into consideration, as being race characters.
Body colour is normally white with a shade of light blue in chinese races and pink in japanese races, visible to the union place of the larva body segments.

2.2. The variability of larva physiological characters

The **moulting**, respectively the moults number, represent one of the most important physiological character of the larva. The primitive races are characterized by 3 moults, while developed races have 4 moults.
The geneticists appreciate that the presence of 3 moults represent the dominant character and the responsibles for hereditary transmition of the moults number are 3 multiple alleles: M^3 and M^5, the dominant relationships being tri>tetra>penta (Tazima Y., 1964).
At the same time, the moulting is controlled by the combined action of the juvenile hormone secreted by corpora allata and the moulting hormone-ecdysone-secreted by the prothoracic gland, both being under the control of activator hormone secreted by neuro-secrethoris cells of the cerebroid ganglions.
The races existing within the sericultural native genetic stock, are characterized by the presence of 4 moults, excepting “Three Molter” race with 3 moults.
The duration of **larval stage** characterizes every race group, being shorter in tropical races (26-28 days), followed by native races (28-29 days) and longer in japanese ones (30-32 days) (Table 3).

Table 3
The variability of larval stage duration and pupation rate

Race groups	Larval stage (days)			Pupation rate (%)		
	Min	Max	Average	Min	Max	Average
Native races	28	29	28.5	90.2	96.6	92.50
Chinese races	28	30	28.6	80.8	93.8	89.14
Japanese races	30	32	30.0	85.6	96.4	92.30
Tropical races	26	28	27.0	90.0	92.6	91.3

Chrysalis transformation percentage (**pupation rate**) - character which reflects the viability state and their capacity of metamorphosis, have been high at the native race group (90.2-96.6%) and inferior at the other groups, as follows: 85.6-96.4% at

the japanese races, 80.8-93.8% at the chinese races and 90.0-92.6% at the tropical races.

3. The variability of cocoon phenotypic characters

3.1. The variability of cocoon morphological characters

The **cocoon shape**, depending on the race group which belongs to, can be:

- elongated with constriction that characterize the japanese races;
- oval elongated with rounded extremities specific to chinese races;
- spherical, elongated without constriction, characterizing also some chinese races;
- spindle, elongated without constriction and with sharp extremities is the specific cocoon shape to the tropical races.

The **cocoon size**, expressed by the longitudinal and transversal axle length, presents a high variability within the sericultural genetic stock.
By group races, the cocoon size varies between the limits presented in Table 4.

Table 4
The variability of cocoon size

Races group	Longitudinal axle (cm)		Transversal axle (cm)		Cocoons/l	
	Max	Min	Max	Min	Max	Min
Native races	4.20±0.131	3.20±0.171	2.26±0.105	1.95±0.031	69±3	65±1
Chinese races	3.60±0.134	3.50±0.135	2.50±0.116	2.08±0.078	42±2	48±2
Japanese races	4.25±0.152	3.77±0.125	2.49±0.135	1.83±0.104	59±5	67±3
Tropical races	3.30±0.141	2.98±0.111	1.90±0.113	1.66±0.107	100±7	85±6
Races average	3.84±0.140	3.36±0.136	2.29±0.117	1.88±0.080	67±4	66±3

3.2. The variability of cocoon technological characters

Cocoon weight (Table 5) present, by race group, the following average values: the minimum value is between 1.445 (chinese races) and 1.632 g (japanese races), maximum value being between 1.709 (tropical races) and 2.361 g (chinese races).
Cocoon shell weight, corresponding to Table 5, has minimum values at tropical races (0.240 g) and maximum value at japanese races (0.520 g).
Silk content (Table 5) represent one of the most important selection's objective. Generally, the japanese races have maximum values (25.06%) on this parameter.

Table 5
The technological parameters of raw cocoon (g)

Races group	Raw cocoon weight (g)		Cocoon shell weight (g)		Silk content (%)	
	Min	Max	Min	Max	Min	Max
Native races	1.604±0.045	2.273±0.066	0.315±0.011	0.504±0.008	18.045±0.712	22.371±0.575
Japanese races	1.632±0.042	2.233±0.092	0.328±0.013	0.520±0.005	19.040±0.518	25.060±0.295
Chinese races	1.445±0.019	2.361±0.092	0.340±0.003	0.482±0.011	16.530±0.602	24.151±0.643
Tropical races	1.469±0.060	1.709±0.050	0.240±0.007	0.369±0.013	16.789±0.622	22.760±0.546
Races average	1.538±0.042	2.144±0.075	0.306±0.009	0.469±0.009	17.601±0.614	23.586±0.515

The **dry cocoon weight** (Table 6) registers maximum values at japanese races (1.250 g) and minimum values at tropical races (0.676 g). The **fiber length** (Table 6), same as dry cocoon weight, has the maximum value at the japanese races (1356 m).

Table 6
The technological parameters of dry cocoon

Races group	Dry cocoon weight X±Sx		Fiber length X±Sx	
	Min	Max	Min	Max
Native races	0.843±0.028	1.163±0.033	1119±22	1324±38
Japanese races	0.870±0.036	1.250±0.035	1037±16	1356±33
Chinese races	0.724±0.028	1.184±0.031	908±15	1236±24
Tropical races	0.676±0.018	0.823±0.026	746±10	950±27
Races average	0.778±0.028	1.105±0.031	952±15	1216±30

Reeling silk registers also maximum values at native races (44.8%) and minimum values at tropical races (38.6%).
Fiber fineness, expressed by metres/g, has maximum values at native races (3241-3764 m/g). Lower performances, but still notables, have the japanese races (3041-3460 m/g).
Notable performances of fiber fineness have the chinese races (3016-3762 m/g). At the tropical races, the fiber fineness is between 2917 and 3090 m/g, being lower to the previous groups.

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Biometric and Biochemical studies on different Hybrids of the Mulberry Silkworm, Bombyx mori L.

Souad, M. Mahmoud[1] and Azza,T. Ashour[2] (1- Sericulture Res. Dept., Plant Protect. Res. Inst., Agric. Res. Center, Cairo , Egypt, 2- Dept. of Economic Entomology and Pesticides, Fac. of Agric. Cairo Univ., Giza, Egypt).

INTRODUCTION

Sericulture is one of the traditional activities in Egypt. However, the sericulture productivity has suffered due to the need of superior silkworm hybrids suitable for the Egyptian conditions.

The recent increase of the domestic and international markets for silk production combined to the deep-rooted national traditions as well as the favorable climatic condition gives a push for developing the sericulture activities in Egypt.

One of the effective methods of increasing cocoon and silk production on national and international levels is the use of highly productive silkworm (*Bombyx mori* L.) hybrids (Petkov, 1984; Hirata, 1985; Brasla and Matei, 1992; Gupta *et al*., 1992; and Kipriotis *et al.,* 1999). Therefore, estimation expressed in respect of different characaters especially the effective rate of rearing (ERR) and cocooning percentage, characters related to disease resistance and healthiness (Prakash 1986). Also, cocoon characters and cocoon crop; characters related to the economic parameters were evaluated.

Because haemolymph is the only extra cellular fluid in insects having diverse functions such as immunity, transport and as the reservoir for the products which are required for nearly every physiological activity of insects (Sowri and Sarangi, 2002). Also the chemical composition of haemolymph is highly variable among the diverse species examined and at different developmental stages of the same species and such variations probably reflect the balance between the synthesis, storage, transport and degradation of structural and functional proteins during ontogeny as well as response to particular ecological and physiological conditions (Florkin and Jeuniaux, 1974).

Proteins have always been an interesting biochemical role in the development, morphogenesis and almost in all intermediary metabolic pathways of insects (Hiramath *et al*., 2006) and since they are the key organic constituents, their role in the compensatory mechanisms of silkworm is vital (Ramakrishna and Jayaprakash, 2007). Also, protein enzymes play a central role in all metabolic processes, in the structure and function of muscles and other tissues. Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects (Horie and Watanabe, 1980).

Hence, the present studies were carried out to reveal some possible biometric differences representing the main biological and economical characters and some biochemical differences in relation to protein, protease and amylase enzymes content among the different hybrids of the silkworm that can be used in the rearing and breeding program at the Sericulture Research Department, in Egypt.

MATERIAL AND METHODS

1- Silkworm:

Six commercial silkworm hybrids; five imported hybrids namely (TK) a Turkish hybrid, (S_1 x H_2) a Bulgarian hybrid; (HBB x D_1) and (HQIX) Chinese hybrids, (155 x 156) a North Korean hybrid and one local hybrid (NBF) as Egyptian hybrid were selected and reared on Kokuzo-27 mulberry leaves.

2- Bioassay:
Each hybrid was reared in mass with three laying and three replicates each up to the 3rd instar and then 300 larvae were maintained in each replication. The rearing was conducted by the standard rearing method of Krishnaswami (1978) at the Sericulture Research Department in Giza – Egypt. Observation on cocoon yield and its economic traits such as mature larval weight, Effective Rate of Rearing (ERR), larval duration, single cocoon, cocoon shell weights, silk ratio percentage were recorded. The obtained data were analyzed by least significant difference according to Snedecor and Cochran (1980).

3- Preparation of haemolymph samples:
At the fifth day of the fifth larval instar, a few larvae from each hybrid were collected for analysis. Larvae were kept at 4-5°C for 5-10 minutes to facilitate the free running of haemolymph. Samples were obtained by cutting the last abdominal leg and the haemolymph was collected in cleaned, pre-cooled vials containing few crystals of phenylthiorea to prevent oxidation of haemolymph. Samp les were centrifuged at 10.000 r.p.m. for 10 minutes to remove haemocytes. Haemolymph were analyzed immediately for biochemical parameters.

4- Biochemical assays:

a- Total protein:
The quantitative estimation of total protein content was carried out using Bradford method (1976).

b- Protease enzyme:
Protease activity was measured by the casein digestion method according to Birk *et al.* (1962).

c- Amylase enzyme:
Amylase activity was determined as described by Ishaaya and Swiriski (1976).
Based on the biological performance, productivity and biochemical analyses, promising hybrids will be used for rearing and also for the breeding programs conducted by the SRD.

RESULTS AND DISCUSSION

I- Biological traits:
The mean values of the examined most important biological silkworm traits are given in Table (1)

1- Hatchability percentage:
It is observed that the egg hatchability is characterized by comparatively height values except the Chinese hybrid HBB x D_1 (98.5 – 87.2%). The highest egg hatchability appeared in the Turkish hybrid (98.50%) which has a significant difference against the Chinese one HQIX (87.2%) and the control (89.00%). No significant differences between the Chinese (HBB x D_1) and the Korean 155 x 156 hybrids (94.0%) for each.

2- Larval duration:
The larval durations of the above mentioned hybrids were in the range of 33-41 days. Both the Bulgarian and Chinese hybrids S_1 x H_2 and HQIX matured early (33 days) followed by the Chinese and Korean HBB x D_1 and 155 x 156 (34 days), while the Turkish hybrid (TK) recorded the longest larval duration of (36 days).

3- Matured larval weight:
Larval weights varied significantly among the hybrids. The Bulgarian hybrid S_1 x H_2 , compared to other hybrids, attained maximum larval weight (2.68 g) followed by the Turkish (TK) (2.59 g) and Korean 155 x 156 (2.59 g) ones and the minimum larval weight was recorded in the local hybrid NBF (2.53 g) followed by the Chinese hybrid HQIX (2.10 g).

4- Effective Rate of Rearing (ERR):
The mean obtained values for silkworm Effective Rate of Rearing (ERR), a biological trait with a marked contribution to the final formation of cocoon output were comparatively high (97.70 – 90.70%) which represent a high adaptability to the rearing condition of Egypt. Among the different imported hybrids, S_1 x H_2 exhibited maximum ERR percentage, 97.70%. Minimum ERR was recorded for the Chinese (HQIX) hybrid 90.70%, while the local hybrid (NBF) recorded 89.68%.

5- Pupation percentage:
Among six hybrids, the highest pupation rate was recorded by the Bulgarian hybrid S_1 x H_2 (100%) followed by the Turkish one (96%). The least pupation rate was recorded for the Chinese hybrid HQIX (89%).

II- Economical parameters:
Data concerning the most important economical traits are presented in Table (2).

1- Single cocoon weight:
A comparatively high increase of the imported hybrids against the Egyptian hybrid was registered in the raw cocoon weight. Its highest value (1.409 g) was found in S_1 x H_2 , the Bulgarian hybrid and the lowest (0.998 g) in the Chinese hybrid HQIX.

2- Single cocoon shell weight:
Variation in average shell weight was found significant among the five hybrids comparing to the Egyptian one (the control). The highest shell weight (0.309 g) was found in S_1 x H_2 and minimum (0.200 g) in the Chinese hybrid HQIX.

3- Silk ratio percentage:
Silk ratio showed significant variations among the hybrids. S_1 x H_2 gave maximum silk ratio (21.93%) and NBF hybrid exhibited the minimum (18.40%).

4- Cocoon yield:
Cocoon yield expressed as Kg of raw cocoons per one box of eggs (20.000 $\pm$ 200 viable eggs) was comparatively high compared to the control (NBF) hybrid.
The highest values of this parameter (26.300 kg), which is largely depend on other biological traits (hatchability %, ERR%, mean cocoon , cocoon shell weights….etc) was found in S_1 x H_2 while minimum cocoon yield was recorded from the local hybrid NBF (18.400 kg).
Similar results were reported by Hosny *et al.* (1994) as they found that the Korean hybrid 155 x 156 was the most suitable hybrid among four hybrids, to rear under the Egyptian conditions. Kipriotis *et al.* (1999) found that the Bulgarian hybrid S_1 x H_2 as one of the tested hybrid in Greece, proved to be promising for the local conditions. Petkov *et al.* (1999) registered the S_1 x H_1 hybrid as a commercial hybrid in Bulgaria.

III- Biochemical assay:

1- Total protein concentration:
The protein concentrations in the haemolymph samples of the 5th larval instar of *Bombyx mori* L. for six hybrids were estimated calorimetrically and presented in Table (3).
Samples of the different hybrids revealed some variations in haemolymph contents. The highest total protein content was found in the haemolymph of the Bulgarian hybrid S_1 x H_2 (52.00 mg/ml) followed by the Korean hybrid 155 x 156 and Chinese hybrid HBB x DT (51.30 and 51.00 mg/ml) respectively. while the lowest concentration was recorded for the Chinese hybrid HQIX (47.40 mg/ml).
The obtained results were supported by Mahadev Kumar *et al.* (2002); Sowri and Sarangi (2002) and Hiremath *et al.* (2006). They reported that the concentrations of protein were higher in bivoltine races at *B. mori* L. silkworms, followed by cross breed and minimum in multivoltine races. Also, Rajannan *et al.* (1994) found that total

protein concentration differed according to races, it was 1-2 fold higher in the bivoltine strain (NB18) than in the multivoltine strain (Pure Mysore). Similarly, Shigmaatsu (1960) reported that protein metabolism is important in the silkworm because of its vital role in the determination of chemical characteristics of silk proteins like fibroin and sericin.

2- Enzymes:

Enzyme activity increased on resumption of feeding after the fourth ecdysis and showed maximum activity on the 4th day followed by a decline to the end of spinning in the silkworm, *B. mori* L. (Abraham *et al.* 1992). Also, they reported that amylase activity in the haemolymph reached maximum in the 3rd or 4th day of the fourth instar and maintained at steady level until ecdysis. Therefore, estimation of enzymes activity were carried on the 5th day of the last larval instar.

2-a- Protease enzyme:

Estimation of protease activity showed that the Bulgarian hybrid S_1 x H_2 was superior among the six hybrids, the optical density of enzyme recorded (109) followed by the Korean hybrid 155 x 156 (54 O.D) then the Egyptian one (50 O.D), while the Chinese hybrid HQIX exhibited the lowest activity of (31 O.D), (Table 3).

The variation range was very wide among the protease activity in the Bulgarian hybrid and the other five hybrids under study.

These results may be supported by the findings of Eguchi (1982) who found clear differences between different strains of the silkworm in the electrophoretic patterns of haemolymph protease inhibitors. He concluded that this will be an important tool for the genetic study of haemolymph protease. Basavaraju *et al.* (1999) reported the peak activity of protease was observd at the middle part of each instar at 25^{o}C. Arundhati-Choudhury *et al.* (2005) noticed that the haemolymph protein profile of the silkworm. *Anthereea assama* Westwood revealed an apparent detrimental effect of the protease on biologically important proteins of silkworm larvae.

2-b- Amylase enzyme:

Analysis of amylase enzyme revealed that the Turkish hybrid TK recorded the highest enzyme activity of 58 µg/g followed by the Bulgarian one (20.99 µg/ml). The Chinese hybrid HQIX showed the lowest activity of 9.63 µg/ml compared to the other hybrids.

These results confirmed the findings of Yokoyama (1959) who reported the presence of two types of amylase activities in the digestive fluid and haemolymph of the silkworm *B. mori* Buonocore *et al.* (1976) stated that in silkworm *B. mori* and many other insect species successful adaptation depends on the level of digestive amylase. Patnaik and Datta (1995) found that 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. Bala *et al.* (2004) stated that the silkworm *Philosamia ricini* showed lower amylase activity compared to *Anthereae assama* silkworm. They attributed the low activity to one of the reasons for the high mortality of the silkworm specially during summer and winter seasons.

It could be concluded that both hybrids, the Bulgarian S_1 x H_1 and the Turkish TK showed remarkable superiority over the other hybrids and can be used for the spring rearing season in Egypt.

Table (1): Biological performance of the imported and local hybrids of the silkworm, *Bombyx mori* L. under Egyptian conditions.

Characters / Hybrid	Hatchability (%)	Larval duration (day)	Mature larval weight (g)	Effective rate of rearing (%)	Pupation (%)
$S_1 \times H_2$	94.70	33	2.68	97.70	100.00
TK	98.50	36	2.59	94.66	96.00
HBB x D_1	94.00	34	2.59	97.40	83.00
HQIX	87.20	33	2.10	90.70	89.00
155 x 156	94.00	34	2.59	97.40	90.00
NBF	89.00	35	2.53	89.68	91.60
F. value	392.00	-	52.52	386.00	637.14
LSD	0.88	-	0.118	0.70	1.44

Table (2) : Economical characters of the imported and local hybrids of the silkworm *Bombyx mori* L. under Egyptian conditions.

Characters / Hybrids	Single cocoon wt. (g)	Single cocoon shell wt. (g)	Single cocoon shell ratio (%)	Cocoon yield per one egg box
$S_1 \times H_2$	1.409	0.309	21.93	26.300
TK	1.366	0.274	20.05	24.500
HBB x D_1	1.113	0.223	20.03	20.600
HQIX	0.998	0.200	20.04	20.00
155 x 156	1.341	0.266	19.83	23.35
NBF	1.145	0.222	18.40	18.400
F. value	19.67	10.20	10.56	-
LSD	0.017	0.012	0.085	-

Table (3): Haemolyph protein content of the six hybrids of the silkworm *B. mori* L.

Hybrid	Total protein concentration (mg/ml)
Bulgarian $S_1 \times H_2$	52.00
Turkish TK	49.60
Chinese HBB x DT	51.00
Korean 155 x 156	51.30
Chinese HQIX	47.40
Egyptian NBF	49.20

Table (4): Enzymes activity of the six hybrids of the silkworm *B. mori* L.

Hybrid	Protease activity OD x 1000	Amylase activity (µg glucose/min/ml haemolymph)
Bulgarian $S_1 \times H_2$	109	59.38
Turkish TK	64	20.99
Chinese HBB x DT	52	12.79
Korean 155 x 156	54	14.95
Chinese HQIX	31	4.63
Egyptian NBF	50	10.06

SUMMARY

Biometric and Biochemical studies were conducted on five imported hybrids from different origins and one local hybrid (as a control) of the mulberry silkworm, *bombyx mori* L .The most important biological and economical traits as hatchability percentage, larval duration, mature larval weight, Effective Rate of Rearing (ERR) and pupation percentage. Cocoon, cocoon shell weights, cocoon shell ratio and cocoon yield were registered as well as biochemical traits as haemolymph total protein, protenase and amylase enzymes were assessed.

Out of the six *B.mori* L. hybrids, the Bulgarian hybrid (S_1 x H_2) showed the best performance with regard to cocoon yield (26.300 Kg./ one box of eggs) and its economic traits namely larval weight (2.68 g.) ERR (97.70 %), larval duration (33 day), pupation percentage (100%) , cocoon weight (1.409 g.) and shell weight (0.309 g.) .

Level of protein content and enzymes activity were different among the hybrids. The highest protein content and protease enzyme activity were observed in the Bulgarian hybrids S_1 x H_2 , while the highest level of amylase enzyme was recorded for the Turkish hybrid (TK) followed by the Bulgarian one S_1 x H_2 .

Data revealed that all the imported hybrids (except the Chinese hybrid HQIX). Showed good performance and can be used for rearing under the Egyptian conditions.

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The use of Ultraviolet Radiation in Silkworm Rearing.

Paschalis Harizanis(*)[1,2], Michael Goliomytis[1] and Marios Tzitzinakis [2,3] (1.Agricultural University of Athens, Laboratory of Sericulture & Apiculture, 2.Sericultural Laboratory of Athens, 3.Hellenic Ministry of Rural Development & Food, Directorate of Animal Production, Department of Apiculture – Sericulture.

ABSTRACT

Ultraviolet radiation has been used to kill or inactivate bacteria, viruses and other microorganisms in air, water, and surface disinfection in hospitals, food processing industries, cold storage rooms, packing material, etc. The UV lamps used were low-pressure mercury vapor lamps that emit radiant energy in the UV-C range, mainly at a wave length of 254 nm. Ultraviolet germicidal irradiation (UVGI) kills or inactivates cells by damaging DNA or RNA inside the cell. A UV disinfection system was used in silkworm rearing rooms to test its effectiveness. For the evaluation of the microbial flora agar plate counts were prepared and at the end of the silkworm rearing, several parameters were estimated such as number of microbial colonies developed in agar plates, larval mortality, cocoon spinning, larval duration, cocoon weight and pupal weight. The most practical use of the described UV light system is to be used only during the first three larval instars.

INTRODUCTION

Ultraviolet (UV) energy has been used for airborne pathogen reduction. It destroys viruses, bacteria, fungi and other pollutants and is used to sanitize rooms and equipment. Ultraviolet radiation is divided into three regions: UV-A: 315-400 nanometers (nm), UV-B: 280-315 nm and UV-C: 100-280 nm. The frequency in the ultraviolet spectrum that best inactivates microorganisms, mold spores and viral contaminants is centered on 254 nanometers (nm), located in UV-C segment (University of Rochester, 2008). Commercially available UV lamps used for germicidal purposes are low-pressure mercury vapor lamps that emit radiant energy in the UV-C range, predominantly at a wave length of 253.7 nm (Green and Scarpino, 2002).

Ultraviolet germicidal irradiation (UVGI) is defined as optical radiation in the shortwave UV-C spectrum capable of killing microorganisms and has been demonstrated to be effective against certain airborne bacteria (Dumyahn and First, 1999). UVGI kills or inactivates cells by damaging DNA or RNA inside the cell. It is inactivating the reproductive abilities of the cell or destroying the cell by inhibiting metabolic action, especially protein synthesis (Schwartz, 1998). Air disinfection techniques are efficient and economic by stand-along, duct or upper-room air units in the laboratory environment (Salie te al., 1995, Scarpino et al.,1998). Three primary factors determine dosage: **a)** Distance between the UVGI lamp(s) and the pathogen.**b)** Intensity of the UVGI lamps and **c)** Duration of exposure to the UVGI lamp(s).

UV lamps are used for killing or inactivating bacteria, viruses and other microorganisms. Typical application examples include air, water, and surface disinfection in hospitals, bacteriological research and pharmaceutical institutions, food processing industries, such as dairies, breweries and bakeries. They are also used for the disinfection of drinking water, waste water, swimming pools, air conditioning systems, cold storage rooms, packing material, etc.

Ultraviolet radiation can be associated with adverse health effects depending on duration of exposure and the wavelength. The adverse health effects that may occur are erythema (sunburn) and photokeratitis. Short-term overexposure to UV radiation can cause erythema and keratoconjunctivitis, skin cancer, melanoma, cataracts and retinal burns (National Institute for Occupational Safety and health, 1972, University of Rochester, 2008).
The Relative Humidity adversely affects the effectiveness of UV radiation especially of values above 80% (Riley and Kaufman, 1972). The moist environment of silkworm rearing rooms can be a perfect breeding ground for airborne fungi and other microorganisms and is interesting to test the usefulness ultraviolet radiation.
Two experiments took place in order to evaluate the possible advantages of the use of UV disinfection system in silkworm rearing. In experiment 1 a comparison was attempted between UV radiation and chemical disinfection. In experiment 2 a combination of chemical and UV disinfection was tested.

MATERIALS AND METHODS

a. Rearing rooms

Two silkworm rearing rooms were operated at 25^{o}C and 75% relative humidity with the help of an air conditioner and a humidifier. The first room was supplied with the UV light system (UV room) for the first 12 days of silkworm rearing and the second one without it (control room). In experiment 1 control room was treated with formalin prior to silkworm rearing. The UV room was not treated with any disinfectant. The room and equipment were just deterged prior to rearing. In experiment 2, both rooms were chemically disinfected by formalin prior to silkworm rearing. The UV room was supplied with the UV light system for the first 14 days of rearing.

b. UV light system

Four TUV 115w PHILIPS lamps were installed in a protected metal tube and at the end of the tube a small ventilator was connected to create air flow. For safety reasons the system was turned off when any work was performed in the silkworm rearing room. The lamps were low-pressure mercury-vapor discharge, consisting of a tubular glass envelope, emitting short-wave ultraviolet radiation with a radiation peak at 253.7nm (UV-C) for germicidal action. The glass filters out the 185nm ozone-forming line. A protective coating on the inside limits the depreciation of the useful UV-C radiation output (Long life lamps). PL-S has a specially adapted starter providing almost instant starting characteristics already built into the lamp base. The useful life is 5,000 hours.

c. Materials

For the evaluation of the microbial flora the following materials were used: a) plate count agar medium (PCA) and plate count RBC (Rose Bengal Chloramphenicol) agar medium, b) Bunsen burner, c) sterilized water, d) 70 % ethyl alcohol solution, e) Ringer solution and f) pipettes of 1ml.

d. The impact technique

For the evaluation of the microbial flora of tools and surfaces in rearing rooms, plate counts methods can be used like the swab technique. For the evaluation of microbial quality of air in the rearing room impact techniques was used. In **impact technique**, agar surface of plate count PCA and plate count RBC agar medium were moved quickly into the air for 3-5 seconds, left open for 3 minutes, and then the lid was put back. To check the UV light system in the UV light room, the two types of plate

counts were open for 10 seconds at the exit of the air flow of the UV lamp system. The plate counts were incubated separately at 25 ^{0}C for 3-5 days. The number of colonies were counted and the microbial flora was identified.

e. Sampling

Experiment 1: Each sampling consisted of 5 plate count agar PCA and 5 plate count RCB agar placed in two positions in each treatment room. Position 1 was above silkworms and position 2 was about two meters away from the place where silkworms were reared. Samples were taken every day at noon from the 1st to the 12th day of rearing.

Experiment 2: Each sampling was consisted of 10 plate count agar PCA and 10 plate count RCB agar. A total of 6 samplings were carried out as it is shown in table 1.

Table 1. The 6 samplings as are shown in the two rearing rooms on the 2nd and 14th day of rearing.

Sampling day	Control room	UV light room	UV light room
2nd day of rearing	Open air	Open air	Exit of UV air flow
14nd day of rearing	Open air	Open air	Exit of UV air flow

f. Silkworm rearing parameters

To check the successfulness of silkworm rearing, several parameters were estimated:

Experiment 1:

1. Number of microbial colonies developed in agar plates.
2. The % mortality (dead larvae at the end of rearing).
3. The % cocoon spinning (larvae that completed cocoon spinning).
4. Larval duration (Time in days requiring from egg hatching to the onset of cocoon spinning).
5. Cocoon weight.
6. Pupa weight.

Experiment 2:

1. Number of microbial colonies developed in agar plates.
2. The % mortality (dead larvae at the end of rearing).
3. The % cocoon spinning (larvae that completed cocoon spinning).
4. Larval duration (Time in days requiring from egg hatching to the onset of cocoon spinning).

RESULTS

Experiment 1

Number of colonies developed in agar plates, in the control and UV rooms, for two positions are shown in table 2. For both agar plates (PCA and RBC) in position 1, above silkworms, number of colonies was significantly higher ($P<0.05$) in the UV room than the control, throughout the experimental period. In position 2, for PCA and RBC agar plates, no differences between UV and control room were determined except for 2,4, 6, 10 and 2 day of age, respectively.

The number of colonies developed in agar plates, in the control and UV rooms by position is shown in table 3. There was a significant effect of position on number of colonies in UV room ($P<0.05$) in most ages. Number of colonies developed in position 1 was greater than that in position 2. On the contrary few differences between position

1 and 2 in the control room were observed. These results indicate that microbial load from the previous rearing was successfully eliminated by chemical disinfection of air and equipment in the control room, but not in the UV room were only the air was disinfected via UV light. As a consequence a greater number of colonies were developed on the silkworm level, in the UV room, than in the control room as shown in table 2. The micro organisms developed on agar plates were mainly fungi, and few yeasts and bacteria.

Table 2. Number of colonies by age, for agar, position and treatment (mean ± std dev).

	PCA				RBC			
	Position 1		Position 2		Position 1		Position 2	
Age	Control	UV	Control	UV	Control	UV	Control	UV
1	8±4,2[a]	144,4±69[b]	10,2±2,6	16,4±6,1	7,8±1,1[a]	144,8±101,8[b]	9,2±4,4	11,6±2,9
2	8,2±1,5[a]	142,5±66,9[b]	6,2±3,0[a]	11,2±3,6[b]	6,4±1,1[a]	126,6±57,5[b]	3,4±1,5[a]	8,6±4,6[b]
3	2,2±1,9[a]	185,8±87,6[b]	3,6±1,5	10,6±8,1	1,6±1,5[a]	96,6±34,7[b]	3±2	4,2±1,5
4	4,8±1,9[a]	49,8±19,5[b]	7,2±1,3[a]	3,8±1,9[b]	4,4±2,9[a]	36±10,1[b]	4±2,4	2,2±1,1
5	1,4±1,1[a]	66,6±22,9[b]	3±1,9	4,6±3,7	1,8±0,8[a]	22,2±4[b]	3,4±1,7	5±2,1
6	7,2±2,4[a]	656,4±174,7[b]	7,4±1,5[a]	4,6±2,2[b]	1,8±1,3[a]	188±58,2[b]	2±1,2	3±2
7	2,4±1,7[a]	195,4±129,1[b]	1,8±1,3	4,6±3,1	1,2±1,3[a]	86,6±40,4[b]	2±1	1,2±0,8
8	3,6±1,7[a]	29,2±11,6[b]	4±3,9	1,2±1,3	2,4±2,1[a]	23,8±11,8[b]	2,4±1,3	1,8±1,5
9	3,8±2,2	6,4±4,5	3,2±1,6	3,6±1,5	0,4±0,9[a]	8±3,4[b]	0,8±1,1	0,8±0,8
10	8,6±6,4	12,4±4,8	12,6±3,4[a]	3±1,4[b]	0,8±0,8[a]	6,4±4,3[b]	1,4±1,5	2,2±3,4
11	11±2,7[a]	61±38,1[b]	9,4±5,9	5±2,1	2,8±1,1[a]	39,4±28,1[b]	2,6±2,7	1,4±1,1
12	4,2±1,3[a]	26,25±10,8[b] 2	±0**	7,5±3,8	0,4±0,6[a]	31±23,5[b]	1,4±1,1	3,4±2,3

*Means within each position column between treatments with different letters as superscripts are significantly different (P<0,05)
** Mean from two equal observations

Table 3. Number of colonies by age, for agar, treatment and position (mean ± std dev).

	PCA				RBC			
	Control		UV		Control		UV	
Age	Position 1	Position 2	Position 1	Position 2	Position 1	Position 2	Position 1	Position 2
1	8±4,2	10,2±2,6	144,4±69[a]	16,4±6,1[b]	7,8±1,1	9,2±4,4	144,8±101,8[a]	11,6±2,9[b]
2	8,2±1,5	6,2±3,0	142,5±66,9[a]	11,2±3,6[b]	6,4±1,1[a]	3,4±1,5[b]	126,6±57,5[a]	8,6±4,6[b]
3	2,2±1,9	3,6±1,5	185,8±87,6[a]	10,6±8,1[b]	1,6±1,5	3±2	96,6±34,7[a]	4,2±1,5[b]
4	4,8±1,9[a]	7,2±1,3[b]	49,8±19,5[a]	3,8±1,9[b]	4,4±2,9	4±2,4	36±10,1[a]	2,2±1,1[b]
5	1,4±1,1	3±1,9	66,6±22,9[a]	4,6±3,7[b]	1,8±0,8	3,4±1,7	22,2±4[a]	5±2,1[b]
6	7,2±2,4	7,4±1,5	656,4±174,7[a]	4,6±2,2[b]	1,8±1,3	2±1,2	188±58,2[a]	3±2[b]
7	2,4±1,7	1,8±1,3	195,4±129,1[a]	4,6±3,1[b]	1,2±1,3	2±1	86,6±40,4[a]	1,2±0,8[b]
8	3,6±1,7	4±3,9	29,2±11,6[a]	1,2±1,3[b]	2,4±2,1	2,4±1,3	23,8±11,8[a]	1,8±1,5[b]
9	3,8±2,2	3,2±1,6	6,4±4,5	3,6±1,5	0,4±0,9	0,8±1,1	8±3,4[a]	0,8±0,8[b]
10	8,6±6,4	12,6±3,4	12,4±4,8[a] 3	±1,4[b]	0,8±0,8	1,4±1,5	6,4±4,3	2,2±3,4
11	11±2,7	9,4±5,9	61±38,1[a]	5±2,1[b]	2,8±1,1	2,6±2,7	39,4±28,1[a]	1,4±1,1[b]
12	4,2±1,3	2±0	26,25±10,8[a]	7,5±3,8[b]	0,4±0,6	1,4±1,1	31±23,5	3,4±2,3

*Means within each treatment column between positions with different letters as superscripts are significantly different (P<0.05)

The greater microbial load determined in the UV room resulted in a worst performance of silkworm rearing than in the control room. As shown in table 4 larval mortality was higher and cocoon spinning was lower in the UV room indicating that, the higher microbial load affected silkworms' performance. Furthermore, cocoon and cocoon shell weight were significantly higher in the control room (P<0.05).

Table 4. Results of the silkworm rearing.

Treatment	No of larvae started rearing	Larvae mortality (%)	Cocoon spinning (%)	Larvae not spinning (%)	Cocoon weight (mean± std dev) (g)	Cocoon shell weight (mean±std dev) (g)
Control	400	10,75	71,25	18	1,458±0,347[a]	0,302±0,079[a]
UV	400	47,5	52,5	0	1,285±0,398[b]	0,275±0,084[b]

Experiment 2

The results of silkworm rearing are shown in table 5. All three parameters (% mortality, % cocoon spinning and larval duration) were lower but did not differ significantly. This can explained because both rearing rooms were disinfected 10 days before silkworm rearing.

Table 5. Results of silkworm rearing.

Rearing room	Number of larvae started rearing	% larval mortality	% cocoon spinning	Larval duration (days)
Control room	800	4.2	91.7	28
UV light room	800	3.8	93.1	28

In the UV light room there was a significant reduction in microbial load. The reduction was higher at the exit of UV air flow. The microbial colonies that were developed in the agar medium after incubation are shown in table 6.

Table 6. Mean number of microbial colonies developed in both types of agar plates.

Sampling day	Control room, open air	UV light room, open air	UV light room, exit of UV air flow
2nd day of rearing	8.4	4.7	3.1
14th day of rearing	14.3	7.6	4.5

The microorganisms that identified in the agar medium were mainly yeasts and fungi (*Alternaria, Dimorphospora, Margaromyces Penicillium)*, and much fewer bacteria (*Bacillus, Streptomyces, Escherichia)*. The yeasts were not identified. The genera of these microbs are characterized as airborne.

DISCUSSION

Results obtained from the present study in show that chemical disinfection of the rearing room and equipment is essential for successful rearing of silkworms. The solely use of UV light for air disinfection does not guarantee the elimination of microbial load from the previous rearing, especially that developed on equipment. As a result, higher mortality, lower cocoon spinning and lower cocoon and cocoon shell weight was observed for silkworms treated with UV light without chemical disinfection prior to the rearing. Room and air chemical disinfection prior to silkworm rearing followed by continuous UV light air disinfection, for the first three critical larval instars, from 1st to the 12th -14th day of rearing, would probably result in a better control of microorganism population and improved performance for the rearing. This hypothesis is tested in the second experiment that follows.

The greater number of larvae that did not spin cocoons in control room was attributed to the extended length of the rearing period in the control room, which was 32 days in comparison with 30 days in the UV room. Cocoons in both experimental rooms were collected by the end of spinning in the UV room. Thus a number of larvae in control room did not have enough time for spinning.

In experiment 2, the results of silkworm rearing showed no statistical difference in % mortality, % cocoon spinning and larval duration. The explanation is that disinfection prior to silkworm rearing is of vital importance.

The identified microorganisms were mostly fungi and yeasts and much fewer bacteria. The UV light is most effective on bacteria than fungi. According to bibliography, most fungi typically require 10 to 50 times more energy to destroy than is required for bacteria. Non of the identified fungi (airborne) in the agar medium are silkworm pests. Several species of *Bacillus* bacteria can infect silkworms but can be easily killed by UV light.

Even though the UV light system was operating on a 24 hours basis (except feeding times) we still had a large number of microorganisms. This is obvious because workers are entering the rearing room with large quantities of mulberry leaves which have a heavy microbial load especially during 4th and 5th instar larvae. Rearing rooms need aeration because during larval feeding carbon dioxide is produced. This production is much higher during 4th and 5th instar larvae. Opening the windows for air

exchange a lot of airborne microorganisms are entering the rearing room and filtering these microbs at the window is not practically possible.
From our rearing experience with UV light, we can suggest that the most practical use of the described UV light system is to use it only during the first three larval instars for the following reasons:

1. The first three larval instars are the most sensitive and that is the period where UV light is more needed.
2. The 3rd larval instar require 1/5 of the 5th larval instar rearing space. For example to rear 20,000 silkworm eggs we need 10 cubic meters space during 3rd instar while 50 cubic meters space for 5th instar. Thus it is easier to disinfect a smaller space with the UV light system.
3. The UV light system is used only for 11 to 12 days instead of 28 to 30 days.

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Combined Effect of Bioinoculants and Medicinal Plant Extracts on Rearing Performance of Silkworm, Bombyx mori L. (PM X CSR2).

R. N. Bhaskar*, Wolktole sori K. R. Shashidhar, S. Sarithakumari and A.N.S Gowda*. Department of Sericulture, UAS, GKVK, Bangalore-65, India. *Sericulture College, UAS, Chintamani.

ABSTRACT

The study reveled that, the leaves raised with bio-inoculants and fortified with medicinal botanical extracts on *Bombyx mori* showed positive influence on rearing performance of PM x CSR2. Application of bio-inoculants, FYM and inorganic fertilizer in combination to the soil inturn feeding of mulberry leaves fortified with botanical extracts showed maximum mature larval weight (22.83 & 27.87 g/ 10), ERR (92.40 & 98.00) and silk productivity (3.28 & 4.08 Cg/day) in the experimental lot were encountered in the standard check in crop I and crop II, respectively. This treatment was either the same or on par with T4 (Bio-inoculants at 20 T/ha/yr *Azotobacter* + 25 kg/ha/yr *A. awamori* + *T. harzianum* + 75 % recommended NP each through chemical fertilizer with each full of recommended K). It was further enhanced through fortification of mulberry with *Psorelia coreleifolia* (1.02 & 1.01; 5.03 & 5.11; 26.25 & 27.52; 28.63 & 30.10g / 10 larvae) for crop I and crop II, respectively. The same trend was seen even in ERR and silk productivity. As it is reflected in the experimental data.

Key words: Bioinoculants, Rearing performance, Medicinal plants, PMxCSR2.

Introduction:

Sericulture is a biotechnological, labour intensive and rural welfare agro-based industry. It is the process and activity of silk production through cultivation of mulberry, rearing of silkworm and reeling of cocoons. India also earns huge amount of hard currency through export of silk products. Therefore, sericulture has a place in planning for rural development in India.

Mulberry (*Morus* sp) is the only food plant and it plays an important role in the growth and development of silkworm and subsequently the production and productivity of cocoons. The continuous production of mulberry for a long time results in gradual reduction in leaf yield and quality. In rece nt days the decline in mulberry yield and quality has been enhanced through use of chemical fertilizers, organic manures and bio-inoculants. Which not only increase the soil health but also increase in the leaf production per unit area. This reduces the cost of production in addition continuous supply of nutrients in such soil as been reported (Krishna and Bongale, 2001). Bio-inoculants are carrier based preparation containing beneficial microorganism in viable form intended for soil, plant growth and increase biological activity in the rhizosphere (Subba Rao, 1998). Leaf quality and quantity influence the growth and development of silkworms as well as cocoon production and quality of raw silk produced. It is true that, nearly about 70 per cent of silk protein produced by mulberry silkworm is derived from protein of mulberry leaves. Thus silkworm should fed with good quality mulberry leaves in optimum quality for successful cocoon production. From these facts, it is very clear that, mulberry leaf plays a predominant role in the nutrition of mulberry silkworm and ultimately the silk productivity. These two factors mainly dependent on the nourishment made through chemical fertilizers, organic manures and bio-

inoculants and fortified with botanicals will have a added effect on the growth and development of silkworm. Therefore the effort has been made in this experiment.

Material and Methods:

To know the effect of individual bio-inoculants and botanicals and their interaction effect PMxCSR2 hybrid silkworm was reared on mulberry leaves raised by the application of bio-inoculants, which were fortified with botanicals. The leaves were fed to the worms through their entire developmental period. However, mulberry leaves were fortified with botanicals only starting from the first feed of the III instar larvae as per the mode and schedule of application. Observations on mature larval weight, ERR and silk productivity were recorded on individual experiments and also interaction effect. Finally the data were analyzed using ANOVA as described by Cochran and Cox (2000).

Treatment details used for field experiment

Notation	Treatments details
T_1	: Standard check: R. NPK* + R. FYM**
T_2	: 75% NP through chemical fertilizers + 25% NP through *Aspergillus awamori* and *Azotobacter* sp. + R. FYM + R. K***
T_3	: 50% NP through chemical fertilizers + 50% NP through *A. awamori* and *Azotobacter* sp. + R. FYM + R. K
T_4	: 75% NP through chemical fertilizers + 25% NP through *A. awamori* , *Azotobacter* sp. and *Trichoderma hazianum* + R. FYM + R. K
T_5	: 50% NP through chemical fertilizers + 50% NP through *A. awamori* , *Azotobacter* sp. and *T. hazianum* + R. FYM + R. K
T_6	: R. NPK only through FYM
T_7	: R. NPK only through chemical fertilizers
T_8	: Control: No application of any fertilizer

Note: *R. NPK: Recommended nitrogen, phosphorus and potash (100:50:50kg/ha/yr),
**R. FYM: Recommended farm yard manure (12 MT/ha/yr), ***R. K: Recommended Potash.

Botanicals used for the preparation of aqueous plant extracts for fortification of mulberry leaves

Notation	Common name	Botanical name	Parts used
P_1	Congress grass	*Parthenium hysterophorus* L.	Leaf
P_2	Nelanelli	*Phyllanthus niruri* Hook. f.	Leaf
P_3	Babchi	***Psoralea coryleifolia***	Leaf
P_4	Water control		
P_5	Absolute control		

Results and Discussion:

Effect of bio-inoculants on rearing performance of silkworm, *Bombyx mori* L.

The analysis of the experimental data on the application of the bio-inoculants coupled with FYM and inorganic fertilizers to M5 mulberry registered significant results on the mature larval weight of silkworm, *B. mori* in both the crop.

However, the mature larval weight was found maximum (22.83 and 27.87 g/ 10) when the batch of worm fed with mulberry leaves from RNPK and RFYM (T1), in crop I and II, respectively. This treatment was on par T4 (21.71 and 27.83 g/10). The lowest mature larval weight (15.58 and 22.18 g/10) was recorded in the control.

The soil application of different source of bio-inoculants, organic manures and chemical fertilizer caused positive influence on ERR and silk productivity of PM x CSR2 in both the crops. However, maximum survival rate of 92.40 and 98.00 per

cent; 3.28 Cg/day was registered in the batches of worms reared by feeding of mulberry leaves raised by the application of RNPK and RFYM (T1) which was on par with T4 (91.30 and 97.71 %); 3.27 Cg/day. The lowest survival rate of 81.08 and 83.14 per cent; 2.16 Cg / day was recorded in control (Table1).

These results are in conformity with finding of Umesh (1999) who opined the conjunctive use of *Azotobacter* and half the recommended dose of N had positive influence on cocoon and silk productivity in mulberry silkworm. According to Sannappa *et al.* (2005), application of FYM at the rate of 20kg/ha/yr + NPK @300:120:120kg/ha/yr to mulberry resulted in higher cocoon yield, silk productivity and ERR then inorganic fertilizer alone.

Effect of botanicals on rearing performance of silkworm, *Bombyx mori* L.

The silkworm fed with mulberry leaves and sprayed with different plant extracts showed significant differences in crop I and crop II, respectively with respect to mature larval weight, ERR and silk productivity. However, the maximum of 28.63 and 30.13 g/10 of mature larval weight; 98.33 and 100 % ERR and 4.040 cg/day silk productivity were recorded when larvae were administered with *Psoralea coryleifolia* leaves followed by *P. nirurii* (27.87 and 28.40 g /10; 97.33 and 100 %; 3.732 Cg/ day) when compare to absolute control (21.91 and 26.96 g/10; 94.00 and 95.33 %; 2.731 Cg/day) (Table 2). Their interaction effect was also found significant with respect to mature larval weight, ERR and silk productivity (Table 3, 4 &5).

These results are in agreement with findings of Patil (1991) according to whom, an increased larval weight can be realized when mulberry leaves were extrafoliated with *T. procumbens* and *P. coryleifolia* and fed to the silkworm, *B. mori*. Further it is also confirmed by Murgesh (2002) spraying of *T. procumbens, T. terrestris* and *P. hysterophorus* resulted in maximum fifth instar larval weight (33.75, 33.74 and 33.56 g/10 larvae) compared to control. Further Shubha (2005) observed better larval weight and survival rate when mulberry leaves were fortified with *Psoralea coryleifolia* and *P. nirurii* compare to without fortification. Higher silk productivity was encountered in *Psoralea coryleifolia* followed by *P. nirurii* in both the crops (I and II). These results are inline with findings of Sreedevi (2003) who reported that, higher silk productivity of 6.68 Cg/ day was recorded for *W. somenifera* followed by *T. cordifolia* and *T. arjuna* (6.21 and 6.12 Cg/day, respectively).

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Table 1: Effect of bio-inoculants on rearing parameters of silkworm, B. mori (PMxCSR2)

Treatments	Mature larval weight(10/g)		Silk productivity (Cg/day)		ERR (%)	
	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II
T_1	22.83	27.87	3.28	4.08	92.40	98.00
T_2	20.70	27.51	2.93	3.67	90.29	96.98
T_3	20.30	26.59	2.60	3.50	87.42	96.58
T_4	21.71	27.83	3.27	3.79	91.30	97.71
T_5	20.54	26.62	2.82	3.55	88.09	96.81
T_6	19.29	25.76	2.49	3.06	86.51	96.12
T_7	19.50	25.69	2.60	2.95	86.61	95.95
T_8	15.58	22.18	2.16	2.33	81.08	83.14
F-test	*	*	*	*	*	*
SEm±	0.506	0.609	0.09	0.06	0.462	0.331
CD @5%	1.536	1.842	0.277	0.175	1.401	1.004
CV (%)	4.373	4.006	5.734	2.982	0.909	0.602

Table 2: Effect of botanicals on rearing parameters of silkworm, B. mori (PMxCSR2)

Botanicals	Mature larval weight(10/g)		Silk productivity (Cg/day)		ERR(%)	
	Crop I	Crop II	Crop I	Crop II	Crop I	Crop II
Parthenium hysterophorus L.	27.67	28.08	3.506	3.505	96.33	98.00
Phyllanthus niruri Hook. f.	27.87	28.40	3.732	3.751	97.33	100.00
Psoralea coryleifolia	28.63	30.13	4.040	4.097	98.33	100.00
Water control	26.51	27.45	2.778	3.189	94.67	96.00
Absolute control	25.91	26.69	2.731	2.933	94.00	95.33
F-test	*	*	*	*	*	*
SEm±	0.090	0.41	0.040	0.1724	0.394	1.08
CD @5%	0.285	1.30	0.127	0.5433	1.243	3.39
CV (%)	0.573	2.55	2.076	8.5420	0.711	1.90

Table 3: Combined effect of bio-inoculants and botanicals on mature larval weight (g/10) of silkworm, B. mori (PMxCSR2)

Bio-inoculants	Botanicals					
	P_1 P	$_2$ P	$_3$ P	$_4$ P	$_5$	Mean
T_1	28.48	28.61	29.98	28.03	27.54	28.53
T_2	27.92	28.26	29.79	27.03	26.52	27.90
T_3	27.52	27.71	29.93	26.62	25.36	27.43
T_4	27.73	28.28	30.41	26.81	26.93	28.03
T_5	27.12	27.57	29.14	26.85	26.36	27.41
T_6	27.04	27.14	28.75	26.49	25.53	26.99
T_7	27.62	27.82	29.32	26.40	26.17	27.47
T_8	24.42	24.69	26.43	24.13	23.59	24.65
Mean	27.23	27.51	29.22	26.55	26.00	
For mean comparison						
	Bio-inoculants		Botanicals		Interaction	
F-test	*		*		*	
SEm±	0.067		0.053		0.149	
CD @5%	0.188		0.148		0.419	

Table 4: Combined effect of bio-inoculants and botanicals on ERR (%) of silkworm, B. mori (PMxCSR2)

Bio-inoculants	Botanicals					
	P_1 P	$_2$ P	$_3$ P	$_4$ P	$_5$	Mean
T_1	98.43	99.25	99.58	95.05	95.25	**97.51**
T_2	97.25	98.25	98.53	95.35	95.35	**96.95**
T_3	97.42	98.42	98.61	94.25	94.32	**96.60**
T_4	97.12	97.98	98.32	95.82	96.77	**97.20**
T_5	97.76	98.73	98.68	94.72	94.73	**96.92**
T_6	96.66	97.67	97.71	94.25	94.03	**96.06**
T_7	97.38	98.39	98.36	94.23	94.38	**96.55**
T_8	90.49	91.42	91.43	90.66	87.26	**90.25**
Mean	**96.57**	**97.51**	**97.65**	**94.29**	**94.01**	
For mean comparison						
	Bio-inoculants		**Botanicals**		**Interaction**	
F-test	*		*		*	
SEm±	0.255		0.201		0.569	
CD @5%	0.716		0.566		1.602	

Table 5: Combined effect bio-inoculants and botanicals on silk productivity (Cg/day) of silkworm, B. mori (PMxCSR2)

Bio-inoculants	Botanicals					
	P_1 P	$_2$ P	$_3$ P	$_4$ P	$_5$	Mean
T_1	3.90	3.92	4.21	3.51	3.36	**3.780**
T_2	3.56	3.67	3.95	3.35	3.28	**3.565**
T_3	3.43	3.51	3.92	3.28	3.17	**3.461**
T_4	3.79	3.93	4.22	3.53	3.36	**3.765**
T_5	3.54	3.62	3.68	3.30	3.21	**3.471**
T_6	3.36	3.42	3.74	3.15	3.00	**3.333**
T_7	3.21	3.32	3.60	2.98	2.88	**3.197**
T_8	2.86	2.94	3.21	2.64	2.54	**2.839**
Mean	**3.457**	**3.541**	**3.817**	**3.218**	**3.100**	
For mean comparison						
	Bio-inoculants		**Botanicals**		**Interaction**	
F-test	*		*		*	
SEm±	0.026		0.021		0.058	
CD @5%	0.073		0.058		0.164	

Some Biological, Technological and Biochemical – Genetic Characteristics of Mulberry Silkworm (*Bombyx mori L.*) Lines established through Insertional Mutagenesis.

Dimitar Grekov[1] and Teodora Staykova[2].
[1]Agricultural University – Plovdiv. [2] "Paisii Hilendarski" University of Plovdiv, Faculty of Biology, Department of Developmental Biology, section of Genetics, 4000 Plovdiv, BULGARIA.

Abstract
Increasing productivity of silkworm is the major and final aim of breeding science. Modern knowledge in the area of genetics, cytology and biochemistry opens wide possibilities for development and introduction of new methods. One of those possibilities is the method of artificial obtaining of mutations.
Ethylmethanesulfonate (EMS), 1.20g relative weight, was used as a mutagenic factor. Nine lines were selected.
The major biological, technological and biochemical-genetic characteristics were studied. Biochemical markers (non-specific esterases) were used for investigating the genetic variability in the studied variants.
Some biological and technological differences of the major characteristics were established. Bi- and triallele polymorphism was detected by PAGE in three of the esterase loci. Different allelic frequencies were found in the separate variants.

Key words: *Bombyx mori* L., chemical mutagens, haemolymph esterase, polymorphism.

Introduction
The **s**ilkworm breeding industry in Bulgaria aims to solve a number of major issues, such as enhancing the selection of individuals displaying the highest productivity, egg hatchability, pupation rate and resistance to diseases.
A number of steps reflect the process of creation of new, highly-productive breeds: initial evaluation of prospective characteristics, creation of lines with desired quantitative and qualitative traits, subsequent analysis of the genetic resources of the newly-created lines, development of methods for the creation of synthetic lines and evaluation of their genotypic and phenotypic characters.
The improvement of the silkworm breeds is dependant on the level of genetic diversity. Isoenzymes have proven to be particularly suitable markers for the study of the inter-breed genetic variability of the silkworm *Bombyx mori* L. (Patnaik & Datta, 1995).
The goal of the present study was to examine some biological, technological and biochemical-genetic characteristics of different lines of mulberry silkworm obtained through treatment with EMS and on this basis to analyze the genetically determined variability.

Materials and methods
Nine lines of silkworm were studied, all of them produced through treatment with ethylmethanesulphonate (EMS) during the larval period. The obtained lines were indicated as HM with numbers from 1 to 9. The basic biological and technological characteristics were subsequently analyzed.

Using 7.5% PAGE the polymorphism of the nonspecific haemolymph esterases of 450 larvae during the 5th instar (50 individuals from each line) was analyzed. Polyacrylamide gel electrophoresis and isolation of haemolymph were carried out according to Stoykova *et al*. (2003) and Staykova *et al*. (2004). The method of Shaw and Prasad (1970) was used to visualize the esterases.

Results and discussion

1. Biological and Technological Characters

Table 1 presents the values of the studied biological characteristics in the selected silkworm types.

The hatchability varies from 90,620% in HM-1 to 94,473% in HM-9. HM-1 manifested the highest variability - 4,18%, the lowest was detected in HM-7.

One of the most important characters is the pupation rate of the larvae. HM -7 manifested the highest value of 89,142%; the variation of this parameter was high – from 22,7% to 33,2%.

It was detected that the cocoon yield had comparatively constant values in all of the studied lines, varying from 27,432 in HM-5 to 28,748 kg in HM-2.

It was observed that the larval duration had comparatively constant values, with a low variation of the parameter.

Table 2 shows the values of the basic technological characteristics.

The shell percentage values were similar in all lines.

The highest value of cocoon weight was detected in HM-7 - 1,826 g. The lowest value was observed in HM-6 – 1,601g.

The filament length is a basic character of particular importance for the textile and related industries. The longest filament length was detected in HM-2 – 1014,2. The variation of this character was low, ranging from 0,17 in HM-4 to 1,80 in HM-5.

2. Biochemical-genetic Characters

Nine fractions of nonspecific esterases belonging to four of the five previously described esterase zones (Stoykova *et al*. 2003) were detected in the haemolymph spectrum of the studied lines.

The results from the present study confirmed the polymorphism established earlier for three of the esterase loci, marked as Bes B, Bes D and Bes E (Stoykova *et al*. 2003). A further analysis of the germplasm of the studied lines was conducted with respect to the allele composition and the frequencies of different alleles. Line specificity was ascertained (Table 3).

The germplasms of HM-2, HM-3 and HM-5 lines manifested the presence of the three alleles in Bes B - Bes B_1, Bes B_2 и Bes B_3 loci (Table 3). Bes B_1 and Bes B_2 alleles were detected in HM-8. In HM-9 only Bes B_1 allele was established, in HM-1 and HM-6 - Bes B_2 , and in HM-7 - Bes B_3.

The results obtained in the present study suggest the presence of triallele polymorphism in Bes D locus with codominant alleles (Bes D_1, Bes D_2 and Bes D_3). Bes D_1 and Bes D_2 alleles were detected in the germplasms of most of the lines (HM-1, HM-2, HM-4, HM-5, HM-7, HM-8 and HM-9). It was observed that only in HM-6 locus Bes D was monomorphous and represented by Bes D_1 allele.

The expression of esterases from BES E zone – only in the spectrum of some individuals, their absence from the spectrum of other individuals, as well as the differences in the intensity of their expression suggest a triallele polymorphism in Bes E

locus with a presence of a null allele (Stoykova *et al*. 2003). Three alleles (Bes E_0, Bes E_1 and Bes E_2) were detected in the germplasm of HM-2. Only two alleles (Bes E_0 and Bes E_1) were present in the studied individuals from HM-1, HM-3, HM-5 and HM-9 lines. In HM-4, HM-6, HM-7 and HM-8 only one allele (Bes E_0) was detected (Table 3).
Sex-dependent expression of haemolymph esterases was not established.

In sum, from the study carried out as detailed above, the following conclusions are made:

- There are differences in the biological and technological characteristics of the studied lines.
- In view of the manifested polymorphism, the studied enzymes - the non-specific esterases in haemolymph are particularly suitable biochemical-genetic markers for analysis of genetic variability.
- There is evidence for certain differences in the germplasms of the studied lines.
- The established genetic variability could be used during selection to improve strains with regard to their economically important traits of quantity and quality. These traits can be expected to be present in the offspring of genetically different parents (Konicheva *et al*. 1975; Egorova *et al*. 1985; Chatterjee *et al*. 1993; Patnaik & Datta 1995).

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Table 1. Some major biological characteristics in the studied lines of *Bombyx mori* L.

Studied lines	Hatchability %			Pupation rate %			Cocoon yield , kg			Larval duration, h		
	$\bar{x}$	$S_{\bar{x}}$	$S_{\%}$	$\bar{x}$	$S_{\bar{x}}$	$S_{\%}$	$\bar{x}$	$S_{\bar{x}}$	$S_{\%}$	$\bar{x}$	$S_{\bar{x}}$	$S_{\%}$
HM-1	90,620	0,869	4,18	88,834	4,501	22,7	28,792	1,424	22,1	738,550	0,539	0,318
HM-2	93,740	0,202	0,94	88,848	4,553	22,9	28,748	1,473	22,9	738,100	1,021	0,602
HM-3	93,820	0,081	0,38	88,876	4,553	22,9	28,736	1,472	22,9	739,400	0,390	0,230
HM-4	93,850	0,048	0,22	89,043	4,562	22,9	28,743	1,473	22,9	738,450	0,574	0,339
HM-5	93,873	0,058	0,27	85,475	6,354	33,2	27,432	2,040	33,2	739,000	0,591	0,349
HM-6	93,990	0,060	0,28	88,999	4,560	22,9	28,442	1,458	22,9	738,650	0,692	0,409
HM-7	94,006	0,238	0,11	89,142	4,568	22,9	28,741	1,473	22,9	738,450	0,491	0,289
HM-8	94,201	0,070	0,32	88,961	4,558	22,9	28,743	1,472	22,9	739,500	0,376	0,222
HM-9	94,473	0,149	0,69	8,884	4,554	22,9	28,746	1,473	22,9	738,900	0,357	0,211

Table 2. Some major technological characteristics in the studied lines of *Bombyx mori* L.

Studied lines	Shell percentage %			Cocoon weight, g			Filament length, m		
	$\bar{x}$	$S_{\bar{x}}$	$S_{\%}$	$\bar{x}$	$S_{\bar{x}}$	$S_{\%}$	$\bar{x}$	$S_{\bar{x}}$	$S_{\%}$
HM-1	48,786	2,448	22,443	1,630	0,001	0,189	1015,9	1,690	0,73
HM-2	47,784	2,448	22,913	1,630	0,001	0,174	1014,2	2,022	0,87
HM-3	47,801	2,450	22,913	1,630	0,001	0,211	1019,5	0,600	0,26
HM-4	47,799	2,449	22,913	1,630	0,001	0,147	1119,1	0,447	0,17
HM-5	45,381	3,374	33,246	1,630	0,001	0,218	1022,9	4,216	1,80
HM-6	47,746	2,447	22,917	1,630	0,001	0,303	1019,0	1,081	0,46
HM-7	47,794	2,449	22,913	1,630	0,001	0,311	1020,4	0,333	0,14
HM-8	47,764	2,447	22,914	1,630	0,001	0,271	1020,1	0,614	0,26
HM-9	47,755	2,447	22,915	1,630	0,001	0,313	1019,4	0,471	0,20

Table 3. Allele frequencies at polymorphic esterase loci in the studied lines of *Bombyx mori* L.

Studied lines	Allele frequency								
	Bes B			Bes D			Bes E		
	Bes B_1	Bes B_2	Bes B_3	Bes D_1	Bes D_2	Bes D_3	Bes E_0	Bes E_1	Bes E_2
HM-1	0	1	0	0.80	0.20	0	0.60	0.40	0
HM-2	0.44	0.22	0.34	0.41	0.59	0	0.71	0.11	0.18
HM-3	0.26	0.24	0.50	0.40	0.52	0.08	0.40	0.60	0
HM-4	0	0	1	0.30	0.70	0	1	0	0
HM-5	0.54	0.31	0.15	0.67	0.33	0	0.46	0.54	0
HM-6	0	1	0	1	0	0	1	0	0
HM-7	0	0	1	0.63	0.37	0	1	0	0
HM-8	0.83	0.17	0	0.67	0.33	0	1	0	0
HM-9	1	0	0	0.52	0.48	0	0.78	0.22	0

Impact of Foundation Cross Male Component on Cross Breed Egg production in South India

S.B.Dandin, Angadi B.S.* and Basavaraja H.K.**
National Silkworm Seed Organisation, Central Silk Board, Bangalore -560 068
*** Silkworm Seed Production Centre, NSSO, Central Silk Board, Chintamani – 563 125**
**** Silkworm Seed Technology Laboratory, Central Silk Board, Bangalore – 560 035**

Abstract
In South India, at present more than 94% of the silk is produced from multivoltine x bivoltine hybrids. Until 1970s only indigenous multivoltine races and their hybrids were reared for commercial silk production. Cross breeding of Pure Mysore with exotic silkworm races like J112, C108 and NN6D though yielded encouraging results in the initial stages, significant improvement was not noticed as there was deterioration of the characters in those races due to varied reasons. The real productivity improvement came up with the introduction of NB4D2 as a male component with Pure Mysore. Due to the high magnitude hybrid vigour of this combination, it became instantly popular and virtually all the traditional hybrids were wiped off form the field.
Consequent to the evolution of productive potential CSR breeds, efforts were made to replace NB4D2 during early 2000s in the southern parts of India .Among the CSR breeds, CSR2 exhibited excellent combining abilities with Pure Mysore and ensured crop stability even under the fluctuating tropical climatic conditions. Thus, the popularly named “Kolar Gold”, (Pure Mysore x CSR2) replaced the ruling hybrid Pure Mysore x NB4D2.
However, owing to repeated multiplications, inadequate rearing facilities of the seed farmers, vulnerable climatic fluctuations and low resistance to pathogens CSR2 very soon appeared to have lost its original genetic potentiality and instigated the present investigations to identify a suitable robust bivoltine male component to replace it. FC2 a foundation cross involving two oval races viz., CSR2 and CSR27 was found to be a promising performer and fit to replace CSR2 and fulfill all the technical requirements of a commercial grainage and economic traits of the reeling cocoons produced. The paper discusses the comparative performance of the male components, its hybrids and their grainage behaviour.

(**Key words** : Foundation cross, Male component, Cross breed egg, Grainage)

Introduction
Perhaps the most significant research break through in the predominantly multivoltine oriented Indian silk industry is the development of cross breed involving multivoltine female and bivoltine male components as parents. Consequent to the demonstration of hybrid vigor in ***Bombyx mori* L.** by Toyama during 1906, there was a spurt of activity in silkworm breeding. The concerted and cumulative efforts of silkworm breeders in both temperate and tropical regions yielded large number of F1 hybrids with well defined genetic, physiological and morphological features which has resulted in assured improved growth rate, better crop stability and acquired resistance to biotic and abiotic stresses (Angadi, 2007). Toyama’s research was repeated by Yonemura, a Japanese scientist who had used this technique during 1921-1923 at Tata Silk Farm, Bangalore, India to produce cross breed eggs for the first time. Similarly, the hybrid vigour principle

was repeated in Maize during 1940s and thereafter rapid success was achieved in crop improvement programmes especially in cross pollinated varieties.

However, until 1970s only indigenous multivoltine races and their hybrids were reared for commercial silk production (Chandrashekariah and Jolly, 1986). Prior to the introduction of Pure Mysore X C.Nichi, the first hybrid exploited commercially in Karnataka state which became popular under poor management conditions of farmers, only pure breeds of multivoltine silkworm races *viz*., Pure Mysore, Nistari, Sarupat, Moria were reared on large scales in South, East and Northern states of India. Cross breeding of multivoltine Pure Mysore with exotic bivoltine silkworm races like J112, C108, and NN6D, was initiated during 1960s. The resultant hybrids though found superior to the pure multivoltine breeds could not stay for long and sustain, as the exotic bivoltine counterparts of the hybrid combinations expressed extensive variability, retracted their voltinism and transformed into multivoltines due to repeated multiplication; continuous rearing under unfavourable tropical conditions and indigent maintenance.

The evolution of KA, NB4D2, NB7 and NB18, new bivoltine breeds in early 1970s by Central Sericultural Research and Training Institute, Mysore and their utilization as male components for the production of multivoltine x bivoltine hybrids ushered a new era, claimed the success and accelerated the spread of silkworm rearing in India. These breeds are being used as male parent for the preparation of F1 hybrids with the indigenous Pure Mysore race in South India and Nistari in Eastern India. The hybrid vigour gained in these hybrids in terms of survival and silk cocoon yield was to an extent of 40% over the mid-parental values (Nagaraju, 2002). Inspite of this breakthrough, the quality of silk produced lingered between the tropical and temperate parental strains involved in the hybrid combinations. However, the fortunate introduction of F1 hybrids of tropical multivoltine female and temperate bivoltine male silkworm strains was instrumental for sustainable sericulture in the country. The new multivoltine x bivoltine hybrid combinations virtually wiped off the traditional multivoltine x multivoltine combination and Pure Mysore x C.Nichi, in major sericulture pockets of Karnataka state. In the history of sericulture, several silkworm breeds and their hybrids have been evolved in the country, out of which only a few stood the test of time and acclimatized to the tropical conditions. During the past two decades, number of multivoltine x bivoltine and multivoltine x multivoltine hybrids *viz*., MY1 x NB4D2, P2D1 x NB4D2, RD1 x NB4D2, G x N, MBDIV x MBDV, MH1 x NB4D2, MU and MG series were developed by CSRTI, Mysore, CSTRI, Berahmpore, KSSRDI, Bangalore, Department of Sericulture, Mysore. These hybrids were tested with the farmers in large scale but failed to get popularity because of occurrence of inherent defects like, diapause eggs, erratic emergence of moths throughout the day (Raghavendra Rao *et al*., 2002). As a sequel to it Pure Mysore x NB4D2 became very popular in the field and flourished for more than three decades.

At this juncture, the incessant efforts of Japanese breeder along with the other Indian counterparts under JICA Project (1992-2001) resulted in the development of CSR2, CSR3, CSR4, CSR5, CSR6, CSR16, CSR17 highly productive bivoltine breeds suitable for rearing in the southern belt of the country during the favorable season (August-February) and robust CSR18, CSR19 breeds for their exploitation throughout the year. One hybrid combination namely CSR2 x CSR4 became very popular and being reared in southern peninsular India. One of the parent *viz*., CSR2 was crossed as trial with Pure Mysore and the results were astonishing (Dandin *et al*., 2004). Taking clue from this, some of the private seed producers resorted to the utilization of CSR2 as male

parent and the resultant hybrid owing to its superior performance became instantly popular. The hybrid was aptly named as “Kolar Gold”. Once the requirement of CSR2 as a male component increased, it necessitated repeated multiplications by various agencies and during the course it appears that the race lost its original genetic potentiality and deteriorated to some extent. Further due its low resistance to all the silkworm pathogens and inadequate care during the rearings, crop failures increased in the filed. Fluctuating climatic conditions also contributed to the poor performance of CSR2. Licenced Seed Producers started complaining about the poor performance of CSR2 as male component as most of them lack proper male moth preservation facilities. In order to overcome these problems being confronted by the seed industry investigation were initiated to find an alternative male component, which can withstand the fluctuating climatic conditions while retaining the economic characters.

Materials and methods

The foundation cross, disease free layings of CSR2 x CSR27 (FC2) produced at CSR and TI, Mysore were procured by NSSO grainages. On the day of hatching the incubated dfls were supplied to the adopted seed rearers. Standard rearing technique advocated by Basavaraja *et al*., (2002) was followed for seed crop rearing. FC2 parent cocoons generated by the ASR’s were procured by following the marketing procedure of NSSO, by four different grainages namely, Chintamani, Vijayapura , Malavalli and Ramanagaram. The desired PM x FC2 disease free hybrid layings were produced by adopting the standard procedure of preparation of multivoltine x bivoltine hybrids as laid down by NSSO.

While the large scale production of PM x FC2 dfls was taken up in the selected four grainages, the data pertaining to the seed crop rearing and the graiange performance was collected and is presented in Tables 1, 2, and 3. And the comparative data of the commercial ruling hybrid and new hybrid developed is given Tables 4.

Results and discussion

Earlier the utilization of various bivoltine breeds/hybrid males for preparation of cross breed seed with PM was studied (Mal Reddy *et al*., 2002). The present study by the authors on the technique of utilizing the foundation cross CSR2 x CSR27 (FC2) male moths in place of CSR2 is based on the basic premises of ;

1. Rearing foundation as a seed crop is easier at farmer’s level.
2. Pupation in foundation crosses is higher with improved cocoon yield.
3. Crop loss is reduced thus avoid seed production risks.
4. Male moths of foundation cross are more vigorous and can be used for multiple matings (2-3 times).
5. Fecundity is better and the egg health is better.

Seed crop rearing performance furnished in Table 1 reveal the superiority of FC2 in all the parameters studied thus making it not only profitable but also technically more fit as seed parent.

FC2 seed cocoon utilization as male component in the grainages registered remarkable advantages as the pairing efficiency is improved by 12.50%, mating capacity by 50%. The gap between the pairs and dfls recovered is reduced by 25.90% which will have a positive impact on the grainage economics. The improved graiange performance of FC2

enables the grainuers to process 12.50% more Pure Mysore seed cocoons as compared to the utilization of CSR2 (Table 2).

Taking advantage of the performance of FC2 seed cocoons four grainages of NSSO, namely Chintamani, Vijayapura, Malavalli and Ramanagaram located in Karnataka state of India totally produced a .quantity 5.50 lakh (equivalent to 11,000 boxes) PM x FC2 hybrids (Table 3) and distributed them to the commercial farmers in the vicinity of respective graianges.

The harvest results of PM x FC2 hybrids were collected on random basis covering about 50% of the dfls supplied. Perusal of Table 4 exhibits the superior performance of PM x FC2 in all the parameters studied especially for fecundity (6.49%), single shell weight (7.14%), shell ratio (4.67%) and the average returns/100 dfls (7.32%).

Enhanced performance of FC2 can be attributed to the considerable amount of its heterotic effects. Recognizing the positive points of using FC2 in seed crop rearing as well as its reproductive performance as evidenced by the data presented, FC2 can be considered as good supplement or an alternative to CSR2 as a male component in the cross breed silkworm seed production centers.

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Table 1. Comparative performance of CSR2 and FC2 as parent race for seed cocoon generation

Parameter	CSR2	FC2	Improvement (%)
Fecundity	422	450	6.63
Cocoon yield/100 dfls (kg)	60.55	62.84	3.78
Pupation rate (%)	85	89	4.70
Average larval period (Hrs)	574	552	3.83
Single cocoon weight (g)	1.62	1.69	4.32
Single shell weight (g)	0.34	0.36	5.88
Shell ratio (%)	20.98	21.30	1.52
Average cocoon rate/kg (Rs) *	265.00	277.00	4.52
Average returns/100 dfls (Rs)	16045.75	17406.68	8.48

* As per proposed proportionate rates based on pupation.

Table 2. Comparative grainage performance of CSR2 and FC2 as male parent with Pure Mysore

Parameter	CSR2	FC2	Improvement (%)
Emergence onset (days)	15	14	6.66
Emergence % of male moths	88	93	5.68
Pairing behaviour	80-85%	90-95%	12.50
Rejection of dfls/100 pairs	8.80	6.52	25.90
Mating capacity (time pairs)	2	3	50.00
Possible ratio of PM : Biv	1:1.75 – 1:2.00	1:2.00 – 1:2.25	12.50

Table 3. Production of PM x FC2 in the southern grainages of NSSO

	SSPC CMY	SSPC VIJ	SSPC MLV	SSPC RMN	Total/ Average
FC2 seed cocoons generated (lakh nos)	2.47	2.67	1.69	1.08	7.91
PM seed cocoons used	5.51	5.01	3.42	1.96	15.90
Pairs obtained (Nos)	227260	200791	136360	78380	642791
DFLs produced (Nos)	196827	171659	116460	64964	549910
% of Pairs	41.28	40.06	39.86	40.05	40.42
% of DFLs	35.75	34.25	34.04	33.21	34.58
Diff of rej	5.53	5.81	5.81	6.85	5.84

(CMY- Chintamani, VIJ-Vijayapura, MLV- Malavalli and RMN-Ramanagaram)

Table 4. Comparative performances of ruling PM x CSR2 and the newly developed PM x FC2 in South India

Parameter	PM x CSR2	PM x FC2	Improvement (%)
Fecundity	493	525	6.49
Cocoon yield/100 dfls	68.900	70.750	2.68
Single cocoon weight (g)	1.847	1.890	2.32
Single shell weight (g)	0.336	0.360	7.14
Shell ratio (%)	18.19	19.04	4.67
Filament length (m)	790	798	1.01
Renditta	8.26	7.99	3.26
Average cocoon rate/kg (Rs)	124.89	130.53	4.51
Average returns/100 dfls (Rs)	8605.00	9235.00	7.32

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Electron Microscope Studies on the Fate of Pathogenic and Non – Pathogenic Bacteria injected into the Hemocoel of Silkworm , Bombyx mori L.

C.S Patil and B.R. Jamuna
Silkworm Pathology Laboratory, Karnataka State Sericulture Research and Development Institute, Thalaghattapura Bangalore-560 062, India.

Abstract
The fate of pathogenic and non pathogenic bacteria injected into haemocoel of silkworm, *Bombyx mori* L with respect to cellular defense reaction was examined over time and relative survival of them were compared. Within minutes of injecting bacteria into hemocoel, profound changes in differential hemocyte count (DHC), total hemocyte count (THC) and a very rapid fall off in hemocyte and bacterial number was accompanied by formation of clumps of hemocytes and bacteria. Although large numbers of both bacterial species were killed/cleared from the hemolymph during the first 6 hrs but the pathogenic bacteria, *Bacillus pantothenticus* eventually overcome the defense mechanism of silkworm and multiplied rapidly in the hemolymph inducing a secondary bacteraemia leading to larval death. In contrast, non-pathogenic bacteria, *Escherichia coli* remained entrapped in the cell aggregates and failed to kill their host. The mechanism of clearance of bacteria from the hemolymph is elucidated through electron microscopic studies and implication of cellular defense reaction against pathogenic and non-pathogenic bacteria injected into silkworm, *B. mori* are discussed.
Keywords: Silkworm, *Bombyx mori*, cellular defense reaction, fate of pathogenic and non-pathogenic bacteria, EM studies and bacterial clearance.

Introduction:
Insects are known to have efficient cellular reactions against invading microorganisms. Several authors have comprehensively reviewed the research on cellular immunity in insects and most of these studies are on insects like *Manduca sexta* (Dunn and Drake, 1983; Horohov and Dunn, 1983; Hurlbert *et al,* 1985) *Galleria mellonella* (Ratcliffe and Gagan, 1977; Ratcliffe and Walters, 1983) and *Hyalophora cecropia* (Boman,1981; Gotz and Boman, 1985) utilizing non pathogenic either heat killed or live bacteria. These studies have not provided information on pathogenic live bacterial infection to the host. Silkworm being economically important insect has been domesticated from centuries and is prone to the attack of several microbes. There are few studies on the defense mechanisms in silkworms (Abraham*, et al.,* 1995; Ashida, *et al*., 1988; Morishima *et al* 1988; Taniai, *et al*. 1997) and these are mostly on elucidating the defense mechanism with respect to non-pathogenic bacteria or bacterial species that do not normally occur in the silkworm environment and none of them focused on the comparisons of pathogenic and non pathogenic bacterial infections.
The knowledge on insect-parasite associations will impart significantly on the development of preventive measures it is also believed that knowing the invasion process of these potentially lethal bacteria will help to yield new therapeutics. Though wealth of information is available on cellular response but there is a need for renaissance of studies to investigate the mechanism governing these processes (Brey and Hultmark, 1998).

In the Present study taking into accounts of these, we have investigated the fate of pathogenic and non pathogenic bacteria injected into the hemocoel of silkworm, *Bombyx mori* L with respect to cellular defense reaction and the mechanism involved in clearance of injected bacteria.

Materials and Methods:

1. Maintenance of silkworms, bacteria and pathogencity test: Bivoltine and multivoltine silkworm larvae required for the experiments were obtained from the disease free stock maintained in silkworm pathology laboratory. Pathogenic bacterium type, *Bacillus pantothenticus* was isolated from the host silkworm, purified and was identified by Microbial Institute, Chandigargh, India. It is a rod shaped, centrally endospore forming gram-positive bacterium. Non-pathogenic bacterium, *E coli* was obtained from Microbiology Department, National Institute of Mental and Neurological Science, Bangalore. The cultures were grown in nutrient broth (Difco Ltd, UK) at 37^{o} C for 24hrs. The total bacterial count in the colony was evaluated using standard plate count method that involves serial dilutions and plating technique.

Pathogencity of *B. pantothenticus* was studied by LD50 method injecting 10 ul of pure bacterial suspension serially diluted upto 10^{-7} from the stock (10^{8} cells/ml) to V instar day one larvae. Mortality of larvae was observed at hourly intervals up to 96 hrs. The *E. coli* was not lethal to silkworm larvae even at 10^{8} cells per larva.

2. Inoculation, bleeding and hemocyte analysis:

Each bacterial culture was centrifuged separately, washed thrice with sterile saline and resuspended in the same and adjusted cell number to 10^{8} cells/ml. Silkworms of V instar I day were swabbed with 70% alcohol and chilled for about 5 minutes and injected with 20 ul of live bacterial suspension and saline injected larvae were served as controls. For studying the hemocytes, larvae were bled directly onto a Neubauer hemocytometer by pricking the base of a proleg with sterile needle and examined under phase contrast light microscope at one-hour time interval of post injection (pi). Types of hemocytes were identified following the identification keys of Akai and Sato (1973) and Gupta (1979b). Total hemocyte counts (THC) and Differential hemocyte counts (DHC) from injected and uninjected larvae were studied every day from III instar day 1 to the end of V instar and calculated following method (Shapiro, 1979b).

3. *In vivo* bacterial clearance:

Silkworms were injected once with 10^{6} cells of each strain of bacteria separately as mentioned earlier. Two hundred micro liters of hemolymph was drawn after 15 minutes, ½ hr, and 1 to 6 hrs with one hr intervals then at, 24 to 48 hrs; and to this 2ml of saline saturated with PTU was added. One ml of this suspension was mixed with 15 ml melted sterile nutrient agar, plated and the bacterial counts were done after 24 hrs of incubation at 37^{0}C. Bacterial clearance rate was deduced by plotting a graph of bacterial count verses time period.

4. Transmission Electron Microscopy:

Hemolymph sample mixed with saline was saturated with Phenylthiourea (PTU) and centrifuged at 1000 rpm for 5 minutes. Supernatant was decanted and hemocyte pellet was washed twice with saline. Hemocyte pellet washed with saline was fixed in 2.5% glyceraldehyde of 0.1M PBS pH 7.2 and post fixed in 1% osmium tetroxide. After the ethanol series, the pellet was transferred to propylene oxide for ½ hr and centrifuged at 5000 rpm for 5 minutes. Then hemocyte pellet was embedded in Epon -Araldite resin and taken thin sections of 75 um for light microscope observation. For TEM studies, ultra thin sections of 70-80 nm were taken using Reichert Jung Ultra microtome

(Germany) and stained with uranyl acetate and Reynold's lead citrate. Sections were observed in TEM (Jeol, Japan) and necessary micrographs were taken.

Results and discussion:

Silkworm is in continuous interaction with environment during its life cycle and faces the risk of infection all the time. As soon as microbes gain the access to the hemocoel, the cellular defense mechanism comes into action within minutes that involves the direct interactions of invader and circulating hemocytes (Patil and Jamuna, 2002). The present study is an elucidation of cellular defense response of silkworm challenged by pathogenic and non pathogenic bacteria for understanding fate of bacteria injected and how silkworm resists/reacts when encountered microbes and their toxins. The hemolymph of silkworm mainly consists of 5 types of hemocytes (Fig. 1). Among them, Granulocytes (GR) and Plasmatocytes (PL) were high in percentage while Spherulocytes (SP) Oenocytoid (OE) and Prohemocytes (PR) were low in numbers. The PL and GR cells comprised the bulk of the total hemocyte population and the total hemocyte number was high in multivoltines compared to bivoltines (Graph 1). According to Kurihara *et al.,* (1992) GR and PL cells are known to take part in cell mediated defense reactions and are referred as immunocytes (Gupta, 1991). THC and DHC studied in uninfected silkworm samples collecting from III instar day 1 to the end of V instar larval period suggests that the THC ranged from 6000-15,000 cells per Cu. mm and it peaked during each moult periods (Graph 2). The increase in THC during each moult was mainly by GR cells and this may be attributed as natural protection strategy of silkworms against microbes as they are more susceptible for infection during moult period since primary structural defenses are weak because of formation of new cuticle and hence the hemocytes increase in large number to act as a back up arsenal (Wago and Ichikawa, 1979).

Experiments were conducted selecting *B. pantothentics* as pathogenic and *E. coli* as non pathogenic bacteria for challenging silkworms. Pathogenicity of *B. pantothenticus* to silkworms was determined by LD50 value. At bacterial concentration of 10^{6} cells/larva, all larvae were killed in 10 hrs of pi while with serial dilution upto 10^{-7} from the same stock, the larvae survived upto 96 hrs and the LD50 was 10^{3} cells/larva. However, non-pathogenic bacteria, *E. coli* with 10^{8} cells/larva did not kill larvae for even 96 hr of pi. These results formed the basis of our experiment to relate pathogenicity and non-pathogenicity of bacteria.

In the present study, it was observed that injection of *E. coli* bacteria into hemocoel caused a rapid decrease in THC and it regained to normalcy by 20 hr of pi while in case of *B. pantothenticus*, although THC decreased initially but there was inconsistency in reaching normal counts. This kind of initial hemocytopenia caused by bacterial injection may be due to involvement of hemocytes in wound healing, increased stickiness of hemocytes to the host tissues (Fay, 1978), cell clump or nodule formation (Ratcliffe and Gagan, 1977). But the recovery of THC may be attributed to either settled hemocytes returning to circulation or production and or release of more hemocytes from hematopoietic tissues.

During the studies, GR cells are the principle hemocytes that played a significant role in recognization of invading bacteria as non-self, trapping and phagocytosis. The first stage of nodule formation was initiated by attachment of bacteria to GR cells followed by degranulation of GR cells that became sticky and formed a localized clot to entrap masses of bacteria. After 2 hr of pi, the PL cells were found to attach and formed loose clumps of 5-6 hemocytes which later increased in size by continued attachment of

hemocytes and bacterial cells resulting in formation of large nodules (Fig. 3). The function of PL cells appears to be cellular signal transductions in initiation and formation of nodules as suggested by Howard *et al.,* (1998).

In vivo bacterial clearance test conducted for non pathogenic bacteria, *E coli* showed a significant drop in free circulating bacteria within 3-6 hr from the hemocoel. More than 95% of bacteria were removed within 6 hr of pi and never increased thereafter even upto 48 hr pi (Graph 3). Light and electron microscopy used to demonstrate cellular defense mechanism with the clearance of bacteria *in vivo* indicate that most of *E. coli* bacteria were found attached to GR cells followed by entrapping and formed a mass of melanized cells. Hourly examination of preparation revealed that most bacteria were within phagocytic vacuoles of GR cells.

But the fate of pathogenic bacteria, *B. pantothenticus* was found markedly different from that of non-pathogenic bacteria, *E. coli*. In case of pathogenic bacteria injected batches, though a bacterial clearance was slow but a significant reduction in viable bacteria was observed *in vivo* between 2-7 hr pi. There after, bacteria reappeared slowly and later there was an exponential increase in bacterial number (Graph 4). Initial interactions between circulating hemocytes and pathogenic bacteria noticed indicates that hemocytes have detected the pathogenic bacteria (invaders), phagocytosed and nodule was formed but unlike non-pathogenic bacteria, the reaction was feeble and not sustainable for long period. Ultra structural studies revealed the destruction of plasma membrane and disruption of GR cells, loss of compactness of organelles resulting degranulation and formation of empty vacuoles. Cytoplasm was vesiculated, plasma membrane was found broken and GR cells begun to lyses by 6 to 24 hr pi. As cells lysed, bacteria were escaping and commonly found in the extra cellular space (Fig. 3). Resurgence of pathogenic bacteria in the hemolymph suggests that the pathogenic bacteria may have the ability to survive and multiply inside phagocytic cells and in the nodules. Cheung *et al*., (1978) suggested that pathogenic bacteria alter their surface antigens so that their progeny are no longer recognized by host's immune response. But in the present investigation, EM studies demonstrate the extensive disruption and lyses of GR cells and escape of bacteria from phagocytosed cells/nodules. Based on this it is hypothesized that pathogenic bacteria being endospore forming, might have secreted toxin that caused hemocyte lyses due to cytotoxic effect and this might have affected the ability of hemocytes to recognize non-self. Later, bacteria multiplied enormously by 10 hr of pi and overwhelmed the hemolymph causing bacteraemia leading to the death of host. The bacterial count just prior to larval death was 10^8 cells per ul of hemolymph and concurrently there was a drastic drop in hemocyte cell numbers.

Thus the present investigation clarifies that onset of cellular defense processes against pathogenic bacteria; *B. pantothenticus* though began approximately at the same time as was for non-pathogenic bacteria *E. coli* but the fate of the *B. pantothenticus* was not the same as that *of E. coli.* It appears that physiological stress experienced by the silkworm larvae due to toxin as well as extensive hemocyte lyses has made cellular processes ineffective in clearing pathogenic bacteria resulted into a general bacteraemia and the death of silkworms despite of an enhanced initial cellular response.

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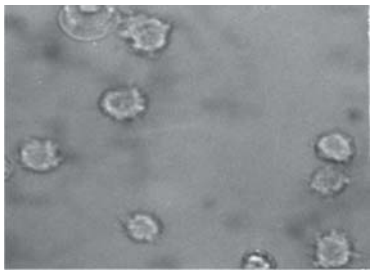
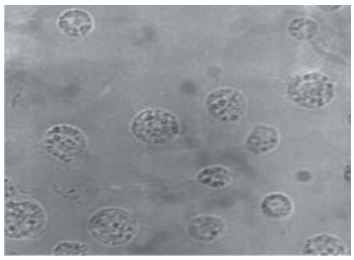
ACKNOWLEDGMENTS:
The authors express sincere thanks to the Department of Biotechnology, Government of India, New Delhi for funding the project.

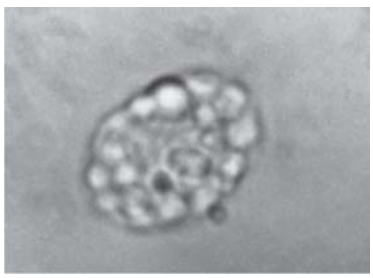
Fig. 1: Hemocytes in uninfected silkworms.



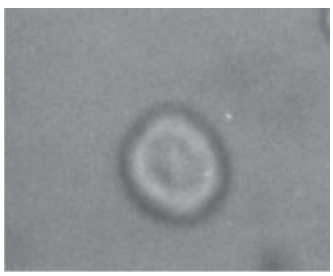
A. Plasmatocyte (PL)



B. Granulocyte (GR)

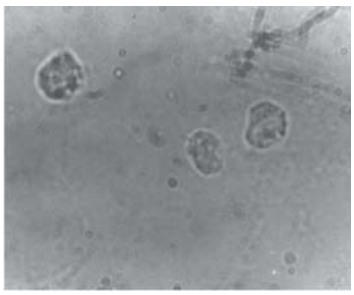


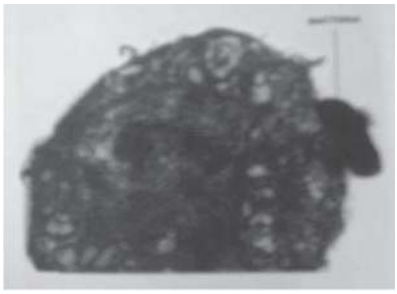
C. Sperulocyte (SP)



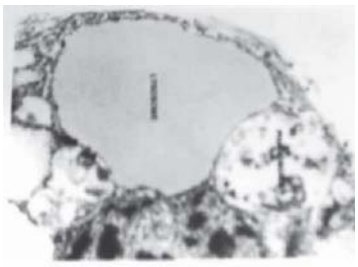
E. Prohemocyte (PR

Fig. 2: Cellular defense reactions after *E. coli* infection.

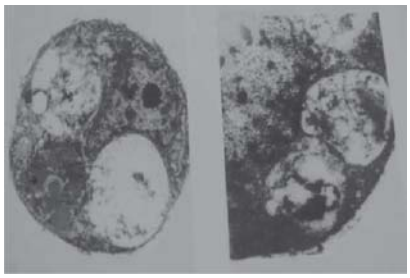




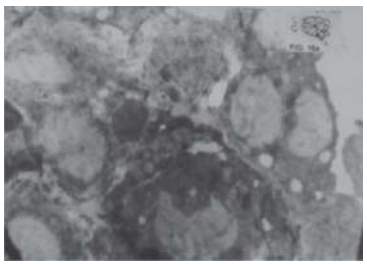
Attachment of bacteria to GR cells, Mag. 15500 X



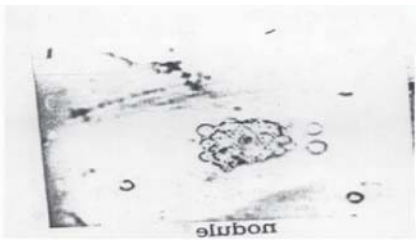
Fusion of lysosome and phagosome, Mag. 22000 X



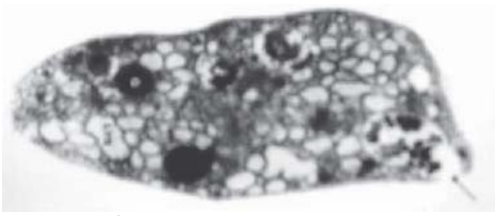
Digestion of phagocytosed materials in phagosome, Mag. 18000 X



Hemocytes clumping Mag. 12000 X

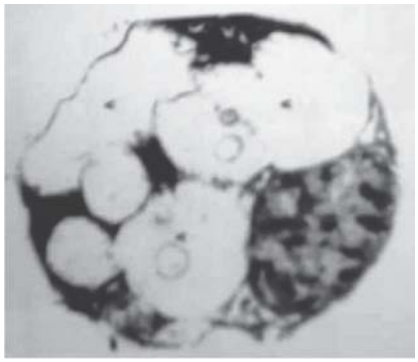


Nodule formation Mag. 12000 X

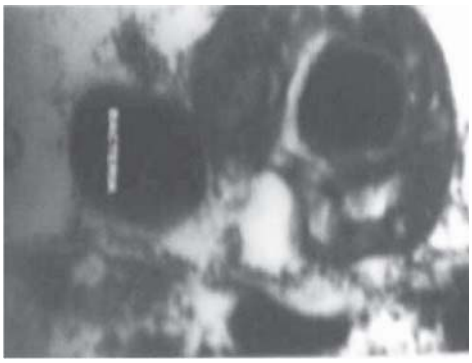


Release of phagocytosed material, Mag. 14000 X

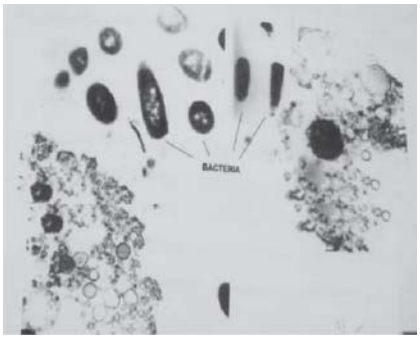
Fig. 3: Cellular defense reactions after *B. pantothenticus* infection.



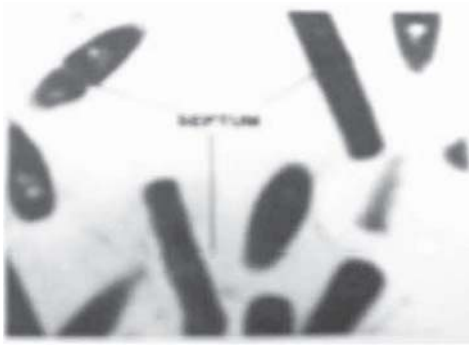
Disruption of GR cells showing empty vacuoles, Mag. 16000 X



Bacteria escaping from membranous whorls of hemocyte, Mag. 24000

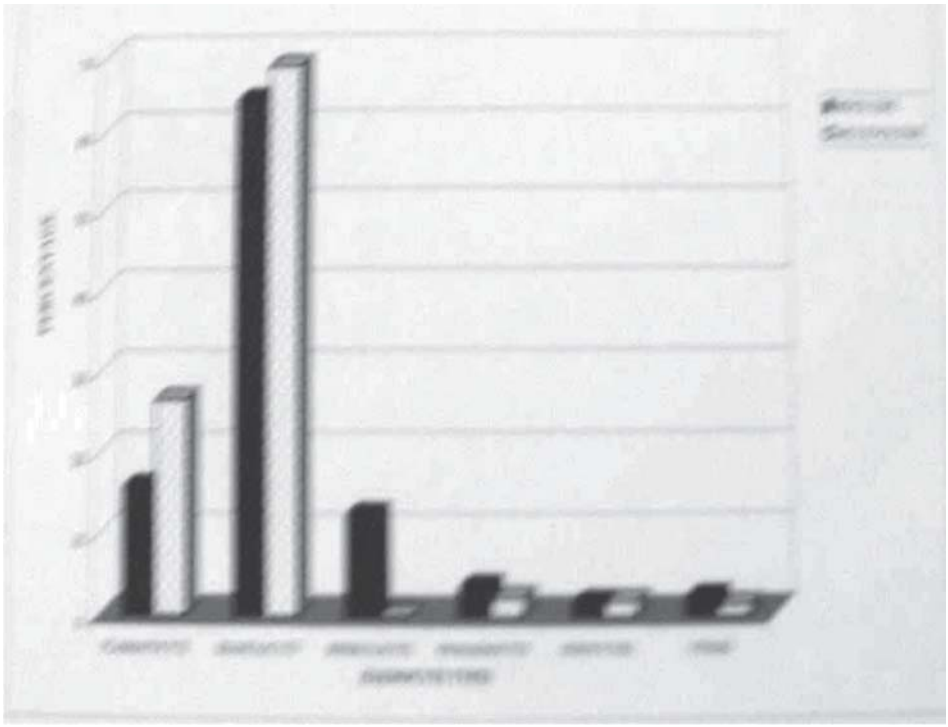


Lysed GR cells with numerous bacteria in the hemolymph, Mag. 25000 X

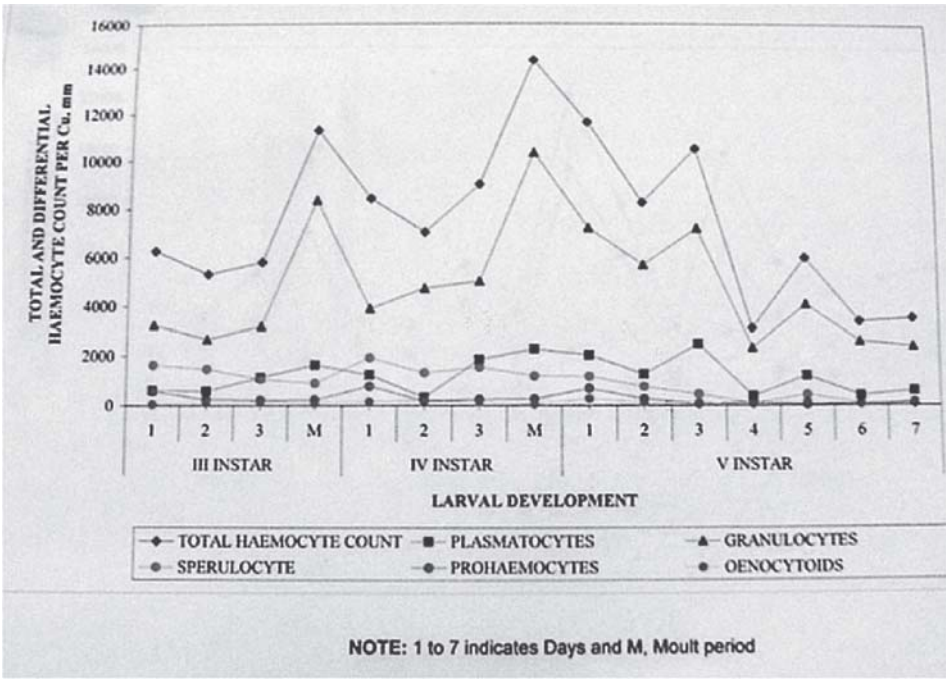


Active bacterial division in the hemolymph Mag. 28000 X

Graph 1: Proportion percentage of different hemocytes in uninfected silkworms.



Graph 2: Total and differential hemocyte counts in uninfected silkworms.



C. BACOLOGY SECTION

Development of gene expression systems in the transgenic silkworm.

T. Tamura, T., Sezutsu, H., Kobayashi, I., Uchino, K., Tatematsu, K., Iizuka, T., and Yonemura, N. (Transgenic Silkworm Research Center, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan).

We have been developed a germ-line transformation system in the silkworm using the transposon *piggyBac* as a vector (Tamura, et al., 2000) and showed that the system is very useful to construct the transgenic silkworms. After authorized the method, many studies using the transgenic silkworms have been reported (Adachi et al., 2006; Inoue et al., 2005; Ogawa et al., 2006;Royer et al., 2005;Sakudho et al., 2007;Tan et al., 2005;Thomas et al., 2002;Tomita et al., 2003;Uchino et al., 2006,2007, Uchlirova 2002). On the other hand, nearly completed genome sequence database of the silkworm has been constructed (Mita et al., 2004; Xia et al., 2004). To understand the function of the genes identified in the *Bombyx* genome database, the development of a suitable gene expression system in the transgenic silkworm and an evaluation of the effect are indispensable.

In this aim, we first studied the feasibility of the yeast *GAL4/UAS* system (Figure 1) in conjunction with *piggyBac* vector-mediated germ-line transformation for targeted gene expression (Imamura et al., 2003). To drive the *GAL4* gene, we used two endogenous promoters that originated from the *Bombyx* cytoplasmic actin (*Bm-A3*) and fibroin light chain (*FiL*) genes. The artificial promoter *3xP3* was also used to test the feasibility of the *GAL4/UAS* system. The EGFP gene under the control of UAS was used to detect the effect of *GAL4* gene. We first tested the function of the *GAL4/UAS* system in the transient expression system of the embryos and then generated transgenic silkworms that carried the *UAS-GFP* construct plus either *GAL4* gene under the control of the actin promoter or *3xP3*. We found that both promoters drove *GAL4* gene expression and that EGFP fluorescence was observed in the targeted tissues of the transgenic silkworms with the *GAL4* and *UAS-EGFP* constructs.

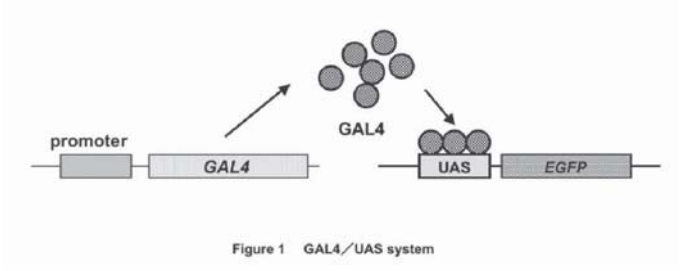


Figure 1 GAL4／UAS system

The silkworms that possessed only the *GAL4* or *UAS-GFP* construct did not show any EGFP fluorescence. We also constructed *FiL-GAL4* lines and the transgenic silkworm with the both constructs, *FiL-GAL4 and UAS-GFP* showed EGFP expression in the posterior silk gland (Figure 2). These results show that the *GAL4/UAS* system is applicable to the transgenic silkworm as the targeted gene expression system.

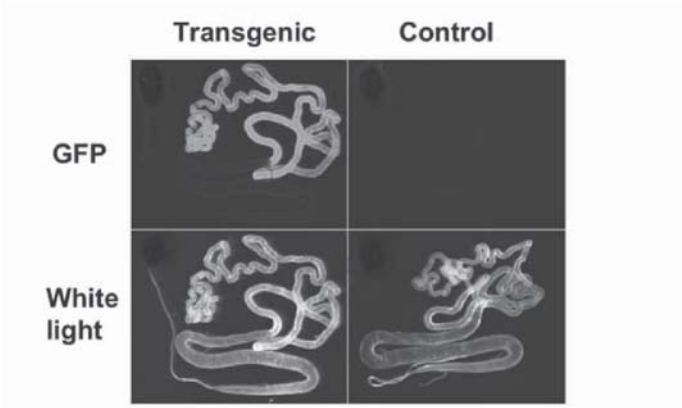


Figure 2 Expression of EGFP in the silk gland of the transgenic sikworm having the constructs of FibL-GAL4 and UAS-EGFP.

Then, we studied the development of the gene expression system that can be conditionally turned on/off the transgene in the silkworm. In the Tet-Off system (Figure 3), the transgene is turned to be on in the absence of tetracycline and to be off in the presence. To examine the system works or not in the silkworm, we have produced the transgenic silkworm possessing tTA gene under the control of *Drosophila* heat shock 70 gene promoter (Dmhsp70-tTA) and EGFP gene with the target sequence of tTA (TRE-EGFP). In the silkworm with the both constructs, the expression of EGFP was observed and it was disappeared by the addition of tetracycline or doxycycline. Furthermore, the expression was recovered when the antibiotic was removed. The results suggest that Tet-Off system works well in the silkworm.

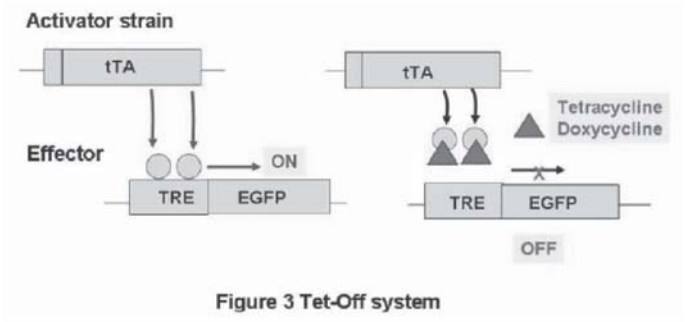


Figure 3 Tet-Off system

Then, we started to construct the enhancer trap system in the silkworm. An

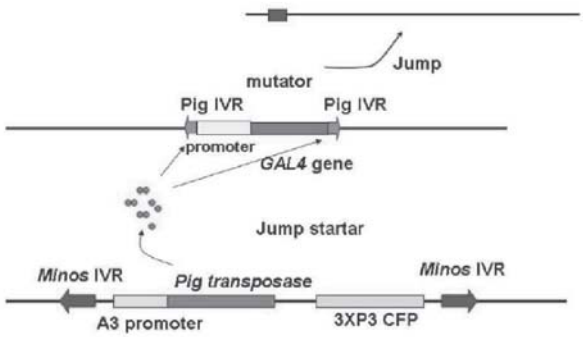


Figure 4 Enhancer trap system developed in the silkworm

enhancer trap system using transposon is very useful tool to analyze the gene function of organisms. The development of the system can be applied for targeted gene expression of the transgene in stage- and organ/tissue-specific manor. The enhancer trap system was firstly developed in *Drosophila* and showed that the system is extremely useful as a tool to obtain the stains controlling an expression of transgene. Recently, the similar system has been developed in the other organisms, like zebrafish, medaka, *Tribolium,* rice, *Arabidopsis* etc and shown that the system is applicable in these organisms.

We previously reported that the transposon *minos* works as a vector in the *Bombyx* (Uchino et al., 2007). In addition, the construct with *GAL4* under the control of *Bombyx* cytoplasmic actin (A3) gene could be used as the mutator (Uchino et al., 2006). Using the tools, we first constructed the *minos* vector to make jumpstarter strain. The vector contains the transposon *piggyBac* transposase gene under the control of *Bombyx* cytoplasmic actin gene promoter and 3XP3DsRed as a marker gene. We produced jumpstarter strains using the germ-line transformation method using the *minos* as a vector and the obtained strains were evaluated their ability to induce the remobilization of the mutator. We obtained four highly active jumpstater strains and showed that the strains can be used to construct the enhancer trap lines. The system developed was shown in Figure 4.Then, we analyzed their expression pattern (Figure 5) and insertion sites. The result

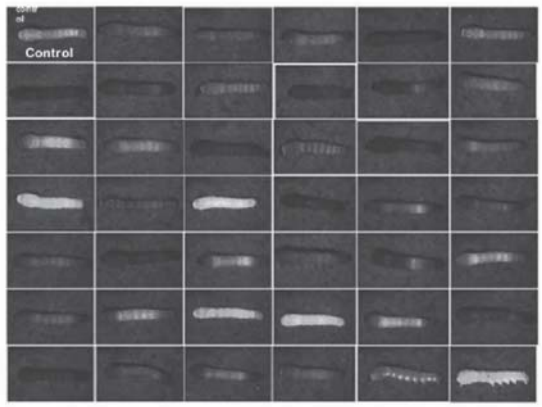


Figure 5 Expression of EGF at the first molting larvae in the enhancer trap lines obtained.

showed that the enhancer trap system developed in this study works very efficiently and that the system can be used to construct large numbers of enhancer trap lines for the analysis of gene function of the silkworm.

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Identification of Multivoltine Mulberry Silkworm Races by Molecular Technology.

Khobkol Sannamvong, Butsara Ravinu and Praveth Sannamvong. The Queen Sirikit Institue of Sericulture Chatuchak Bangkok 10900, Thailand.

ABSTRACT

Besides Phenotype, morphology and agriculture characteristics of mulberry silkworm, molecular technology is more reliable method to identify and classify mulberry silkworms. This study used Amplification Fragment Length Polymorphism (AFLP) with 10 primers (AAC-CAC AAG-CAT ACA-CAA ACC-CAG AGC-CAA AAC-ACT AAG-ACC ACA-ATG ACC-AGG and AGC-ATG) to identify 20 races of mulberry silkworm samples. The results indicated that DNA finger print profiles of the samples were different and produced in the number of 626 DNA bands. These DNA bands had the size in the range of 118-700 bp. The number of DNA bands at the scoring point was 183 bands being 35 bands as monomorphic bands. The others were polymorphic 148 bands or 80.87 percent at every AAC-CAC and ACA-ATG primers.

KEY WORDS : mulberry silkworms, Multivoltine, AFLP, DNA, Molecular technology

INTRODUCTION

For maintenance and preservation of silkworm is the main role of silk breeding for getting the qualitative and quantitative to the farmers. *Bombyx mori* is a good model for solving a broad rang of fundamental biological problems. In the past, the characterization of *B. mori* strains is mainly based on morphological characteristic such as larva skin marking, body shape, and cocoon colour.

At present DNA marker technology particularly PCR based marker such as randomly amplified polymorphic DNA marker (RAPD), amplified fragment length polymorphism (AFLP) have augment the marker resources for genetic analysis of a wide variety of genomes.But because of the tropical region, there are three different silkworm strains of the country based on agriculture characteristics recommended by The Queen Sirikit Institute of Sericulture.These strains are polyvoltine (multivoltine), bivoltine hybrid and multivoltine x bivoltine silkworms. However, based on molecular level, the identification of silkworm races has not been established. Khobkol et al (2541) had studied silkworm races of UDA, NB18 SKN1 x KaewSakol and Kaewsakol x SKN1 with 5 primers OPl-12 OPN-05 OPU-10 OPU-18 and OPX-12 by using molecular biological technology. Duverney et al (2006) had studied 11 Chinese and 12 Japanese silkworm strains and found the molecular markers and the productivity characters were correlated by multiple variance analysis. The analysis permitted the identification of molecular markers associated with the cocoon weight or the shell weight separately. Yuan-De et al (2001) studied an amplified fragment length polymorphism map of the silkworm. They found that of the 545 polymorphic markers, 356 were assigned to 30 linkage groups. The number of markers on linkage groups ranged from 4 to 36. Nagaraji et al (2002) studied on BAC libraries being constructed and used to make large scale physical maps with marker based on ESTs as framework anchors. They provide a foundation for

identification of gene function, gene and chromosome evolution, and comparative genomics. Muwang et al. (2005) studied genetic diversity among silkworm germplasms revealed by using simple sequence repeat markers (SSR markers)and found the average value for each SSR locus ranged from 0 to 0.60.The mean polymorphism index content was 0.66.

Hence, this study was set up to identify mulberry silkworm (*Bombyx mori* Linn.) at molecular level of Thailand using Amplification Fragment Length Polymorphism (AFLP) with 10 primers (AAC-CAC AAG-CAT ACA-CAA ACC-CAG AGC-CAA AAC-ACT AAG-ACC ACA-ATG ACC-AGG and AGC-ATG) to identify 20 races of mulberry silkworm samples.

MATERIALS AND METHODS

MATERIALS

Number of polyvoltine silkworm 16 races as SR4(A1), BR.10(A2), SR5 (A3),NK4.(A4), NK5.(A5), PC21.(A6), Nonluasri (A7), NangLai (A8), Nang Noi (A9), Kaew Sakol(A10), NangLuang(A11), UD03(A12), UD05 (A13), Samrong1(A14), KaKi1(A15), KaKi2(A16),
Number of Bivoltine silkworm 4 races as SKN1(A17), UDB(A18), UDI0(A19), and NB18(A20)
Rearing house with other instruments
Laboratory with Gel Electrophoresis, UV transilluminator

METHODS

1. To extract purified DNA from posterior silk glands
2. Polymerase chain reaction (PCR)
3. Gel Electrophoresis: Polyacrylamide Gel Electrophoresis: to evaluate DNA size and its umber of each primer
4. To evaluate Cophenetic correlation(r), Phylogenetic Tree and their similarity of 20 silkworm races

TIME AND PLACE

TIME: October 2005 – August 2006
PLACE: The Queen Sirikit Institute of Sericulture

RESULTS AND DISCUSSION

The number of DNA bands and their size of both silkworm races (Polyvoltine and Bivoltines) evaluated by AAC-CAC primer were found that there were 30 DNA sizes in the range of 138-574 base pairs (bp) as 138, 152, 155, 157, 165, 168, 170, 176, 179, 184, 185, 189, 196, 198, 204, 229, 231, 243, 247, 253, 253, 271, 283, 298, 315, 325, 332, 359, 364, 444, and 574 base pairs. The DNA bands with the size of 574 bp were found in 17 silkworm races with the exception of A2, A14 and A15. Also, the DNA bands with the size of 271 bp were found in only A1 and A2. Whereas, the DNA bands with the size of 189 bp were not found in A6, A7 and A8. Of 20 silkworm races were found in the monomorphic bands had the DNA size as 157, 247, and 332 bp (Fig 1).

For using AAG-CAT primer, there were DNA bands of silkworm races in the range of 142-521 bp with 23 sizes as 142, 144, 148, 154, 159, 168, 173, 181, 186, 189, 196, 198, 204, 206, 219, 246, 257, 262, 270, 304, 469, 475, and 521 bp. The DNA bands with size of 144, 159, 181, 186 189, 257 and 529 bp were found in 20 silkworm races (Fig1).

For ACA-CAA primer, it was found that the DNA bands with the size in the range of 159-396 bp had 24 bp as 159, 162, 167, 169, 180, 183, 186, 194, 198, 200, 214, 221, 229, 235, 243, 246, 266, 274, 294, 303, 309, 322, 344 and 396 bp. The size of DNA band 348 bp was found in 20 silkworm races. Of the size 332 bp was found in 18 silkworm races but was not found in A1 and A2. While, the DNA band size of 159, 162, 194, 266 and 396 bp were found in 20 silkworm races as monomorphic band (Fig 2).

For ACC-CAG primer, it was found that the DNA bands size range between 140-681 bp comprised 11 bands were 140, 142, 180, 185, 217, 229, 267, 283, 466, 541 and 681 bp. But the DNA bands with the size of 681 bp were not found in A1, A15 and A16. Whereas, the DNA band with 142 bp were found in 20 silkworm races. For the DNA band with the size of 229 269 and 541 bp were found in 20 silkworm races as monomorphic band (Fig 2).

For AGC-CAA primer, it was found that the DNA bands range between 163-608 bp comprising 11 bands as 163, 171, 175, 203, 216, 272, 308, 323, 329, 598 and 608 bp. Of the DNA band 323 bp was found in 19 silkworm races but was not found in A8. Whereas, the DNA band with the size of 203 bp were found in 20 silkworm races. However, the DNA band 175 bp was found in 20 silkworm races but was not found as monomorphic band (Fig 3).

For AAC-ACT primer, it was found the DNA band rang between 141-421 bp comprising 19 bands as 141, 145, 148, 150, 157, 165, 167, 187, 191, 194, 198, 220, 230, 242, 257, 311, 380, 398 and 421 bp. The DNA band of 165, 194, 220, 311 and 398 bp were found in 20 silkworm races as monomorphic band. T he DNA band 167 bp was found only in A11, whereas, the DNA band with the size of 191 bp were found in 20 silkworm races (Fig 3).

For AAG-ACC primer, it was found DNA band rang between 142-321 bp with 15 band sizes as 142, 148, 160, 177, 180, 185, 217, 221, 231, 250, 277, 280, 285, 310 and 321 bp. The DNA band size 233 bp were found in 20 silkworm races. While, the DNA band with the size of 148 bp were found in 18 silkworm races but were not found in A1 and A2. The DNA band with the size of 160, 180 and 250 bp were found in 20 silkworm races as monomorphic band (Fig 4).

For ACA-ATG, it was found DNA band with the size range between 143-472 bp comprising 31 DNA band as 143, 144, 146, 150, 156, 161, 164, 166, 171, 172, 176, 183, 188, 196, 198, 207, 269, 272, 291, 306, 309, 332, 392, 361, 369, 373, 385, 396, 450, 461, and 472 bp. The DNA band with the size 396 bp were found in 18 silkworm races but were not found in A15 and A18. The DNA band with the size of 361 bp and DNA band with the size of 164 bp were found in 19 silkworm races but were not found in A1. The DNA band 143, 144, 176 and 196 bp found in 20 silkworm races as monomorphic band

For ACC-AGG,the DNA band with the size between 143-304 bp comprising 10 DNA band as 143, 153, 155, 161, 172, 174, 219, 261, 217 and 304 bp. The DNA band with the size 304 bp were found only in A6 and A16. The DNA band 277 bp found in 20 silkworm races were momanophic band (Fig 5).

Fig 6 Showed phylogenetic Tree of 20 silkworm races.
Fig 7 Showed the distribution of silkworm race samples by using cophenetic correlation (r) = 0.94
Table 1 showed the number of DNA band of each primer 10 primers). The number of DNA band found in the range of 700-140 bp were 388 bands. The number of DNA band which scored was 209. The number of DNA band which was polymorphism was 161 band.
Based on genetic similarity as simple matching, it was found that all 20 silkworm races had closely genetic results among them. It was indicated that there was genetic similarity index (GSI) in the range of 0.5738-0.9454.
The dendrogram was constructed based on UPGMA (Unweighted pair group method on the basis of arithmetic average). The silkworm races were identified as Group A which the GSI was in the range of 0.6284-0.9126. In this group could be divided into 3 subgroups as:
Subgroup 1 there were 3 silkworm races as A1, A2 and A15 which A1 and A2 had closely genetic similarity index as 0.9126.
Subgroup 2 there were 13 silkworm races as A3, A4, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14, and A16. It was found that A12 and A13had the GSI as 0.9126
Subgroup 3 there were 2 silkworm races as A17 and A19 with GSI as 0.9454 which were the same races.

CONCLUSIONS

From the analysis of silkworm genetic using NTSY Spc 2.10p program similarity coefficient as simple matching cooperated with morphology physiology and agriculture characteristics for mulberry silkworms' identification, it was the most precision to identify silkworm races (both Polyvoltine and Bivoltine). Hence, that was very useful for breeding programe to analyze genetic distance and to identify silkworm varieties. All that could be improved silkworms breeding of the country.

ACKNOWLEDGMENTS

We would like to thank Dr. Somwong Trakoonrung, the director of Genetic Engineering Center, Kasetsart University for helping us on the all laboratory technologies. We also thank Dr. Preeya Wongsomneuke Biology Department, Faculty of Science, Khonkaen University, for helping us on data analysis by using the program of NTSYSpc2 and Dr. Somphob Jongruaysup for discussing some sessions of this paper.

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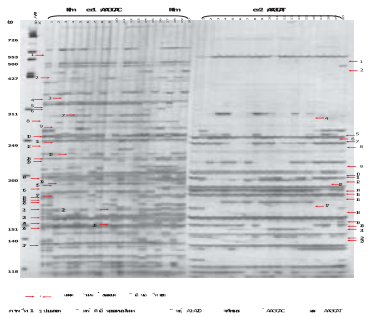

Fig. 1 DNA markers of mulberry silk worm varieties A1-A20 obtained from AAC-CAC and AAG-CAT primers

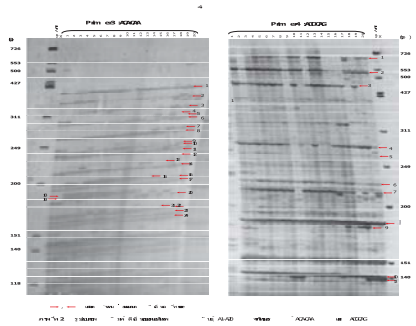

Fig. 2 DNA markers of mulberry silk worm varieties A1-A20 obtained from ACA-CAA and ACC-CAG primers

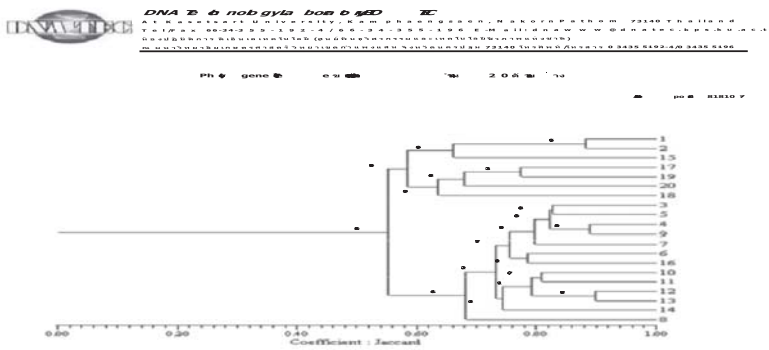

Fig. 6 Showed phylogenetic Tree of 20 silkworm races.

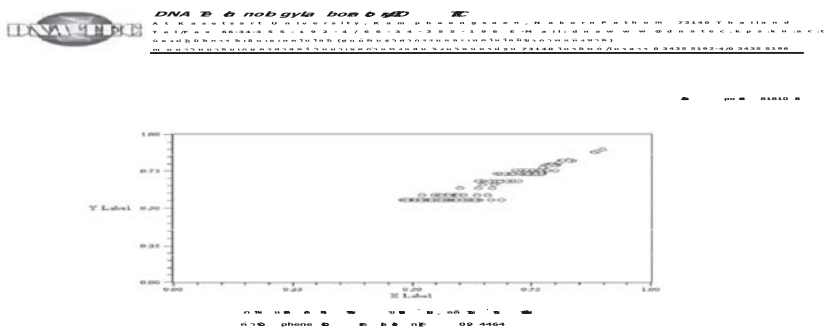

Fig. 7 Showed the distribution of silkworm race samples by using cophenetic correlation (r) = 0.94

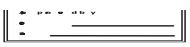

TABLE.1 DNA bands which found at each primer

DNA BANDS WHICH FOUND AT EACH PRIMER			
PRIMER	DNA BANDS (SIZE 700-140 bp)	NUMBER OF SCORED DNA BANDS	NUMBER OF DNA BANDS AS POLYMORPHISM
AAC-CAC	60	30	27
AAG-CAT	34	24	16
ACA-CAA	41	29	23
ACC-CAG	38	15	11
AGC-CAA	25	17	15
AAC-ACT	26	20	14
AAG-ACC	23	18	12
ACA-ATG	50	35	29
ACC-AGG	41	11	8
AGC-ATG	50	10	6
	388	209	161

Development of a generally applicable culture medium for development of insect cultured-cell lines.

SHIGEO IMANISHI, Genebank, Division of Genome and Biodiversity, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Ibaraki 305-8634 JAPAN.

Introduction

Cultured-cell lines are very useful materials for research in molecular biology. Over 500 insect cell lines have been established over the past 40 years. However, most of these cell lines were established using restricted tissues from insects. General culture techniques are not well developed for use with a wide range of insect species and insect tissues. Moreover, it takes over six months to establish cultured-cell lines. Success or failure of cultured-cell line establishment depends several factors including the culture media composition. Here a novel culture medium is described. This new culture medium can easily increase cultured-cells from several kinds of insect species and different insect tissues. The use of this new culture medium using *Bombyx mori* to develop cultured-cell lines is described.

Methods

The new MX medium is being registered as a patent in the U.S. (Patent number 013090/0066). The primary culture was developed using tissues derived from the embryo, ovary, testis and fatty bodies. Primary culture requires approximately six months at 25^{o}C. During that time half the medium is changed every two weeks. The population of cells gradually increases to a point when there are sufficient cells for subculturing. A cultured-cell line is only declared after subculturing over 50 times with cells displaying stabile cell characteristics.

Results: The MX medium composition (MX30) includes 30% by volume of Fetal Bovine Serum (FBS) in the MX medium, and this accelerated both the cell migration from several kinds of *Bombyx mori* tissue and their multiplicationt. This medium shortened the primary culture period by several months compared with other media. Finally, cell lines were established from fatty body, ovary, testis and embryo tissues of *Bombyx mori*. This media can be applied for the establishment of other insect species' cultured-cell lines, too. Some *Bombyx mori* cell lines developed are described below.

One cell line (NIAS-Bm-Ke1) is being used as a large-scale culture *in vitro*. This cultured-cell line can be maintained in a serum-free and low protein medium and can be used for expression of foreign genes using the Baculovirus gene expression system. However, *Bombyx mori* nucleopolyhedrovirus(BmNPV) does not infect cells without a silkworm serum. When 0.1% volume serum was included in the medium, the cells formed many *Bombyx mori* nucleopolyhedrovirus crystals in cells. A new Baculovirus gene expression system by using this serum-free cultured-cell line is being developed.

A plant RNA virus was discovered for the first time in the cytoplasm of a *Bombyx mori* cultured-cell line. It was named BmMLV, because the virus belongs to Tymoviridae order, Maculavirus family, and had been discovered from silkworm. BmMLV has been confirmed in most cultured-cell lines except NIAS-Bm-VF cultured-cell line.

Cardinium living in the body of *Ixodes scapularis* and *Wolbachia* in the body of *Laodelphax striatellus* were found to infect the newly established cultured silkworm cell line NIAS-Bm-aff3 of fat body origin. A microarray study has revealed that *Cardinium* can be infected with the cultured cell and induce the expression of the immune

response genes encoding lebocin, lysozyme, and gloverin of the host cell. On the other hand, the genes expression has not been increased in the cell of *Wolbachia*.
Inducement activity of the immunity inducement gene by peptidoglycan (PGN) in NIAS-Bm-aff3, a cell line derived from silkworm fat body, was studied. Depending on PGN type the induced activity differed. Similarly, an increase in the amount of the transcript and the amount of Calreticurin (CRT) that belonged to the endoplasmic reticula chaperonin was seen in this cell line by processing with ripoporisaccarid (LPS).
The cell lines were processed with ecdyson, and insect molting hormone. Subsequently, the ecdyson-related genes (*BmE75A, BHR3*) were confirmed in SES-BoMo-C129 and NIAS-Bm-oyanagi2, silkworm cultured cell lines, by RT PCR. The induced genes were strongly expressed by the interaction of ecdyson and a juvenile hormone analogue. Also, cuticular protein genes *BmWCP2* and *BmWCP10* were expressed in those cultured cell lines at the same time.

Conclusions
The newly developed cell lines described here are promoting the development of insect molecular research. Cultured-cell lines from the active genebank collection can be distributed under a Biological Materials Transfer Agreement (MTA) of National Institute of Agrobiological Sciences (NIAS). *(http://www.nias.affrc.go.jp/)*

A red fluorescent lipocalin (Polycalin) ChBP (Chlorophyllid A Binding Protein), from the midgut of *Bombyx mori* L.

Bernard Mauchamp, Unitı Nationale Sıricicole/IN RA, 25 quai J.J. Rousseau, 69350 La Mulatiθre, France.

Recent progresses in proteomic and genomic lead us to develop studies on proteins of the silkworm midgut. The midgut is the gate for infection of silkworms after oral ingestion of pathogens. A protein called previously RFP (Red Fluorescent Protein) was described as possessing antiviral activity against BmNPV, but no molecular characteristics were available. We focussed our studies on the determination of its amino acid sequence and the nucleotid sequence of its gene.

Protein after extraction was purified on 2D gel electrophoresis; the fluorescent spot was trypsinized and obtained peptides analized by mass spectrometry. The amino acid sequence of several peptides was determined. Some of these sequences were blasten in *B. mori* EST. From total RNA and RT-PCR with specific primers we established the total cDNA sequence of the protein, then its deduced amino acid sequence. The cDNA encoded a protein of 2721 amino acids (302kDa).

In addition, WGS gene Bank blast allowed to list the contigs BAABO1026110, AADKO1000800 and AAKO1004726.1, and obtain informations on the structure of the genomic sequence characterized by a large number of introns (45) and exons(46).

This protein is a protein of the Lipocalin family, but its size and its arrangment are very new. This protein has 15 lipocalin structures each one characterized by a well conserved tertiary structure We introduce the notion of polycalin (pentadecacalin) and called it ChBP since the ligand is the chlorophyllid, product of degradation of the chlorophyll by chlorophyllase.

D. NON-MULBERRY SECTION

Cluster Analysis and Community similarity in certain Divergent Ecoraces of Indian Tasar Silkworm, Antheraea Mylitta Drury.

***M. Prasad, *A. Dutta, **S.K. Gangwar, **M.K. Sinha and **B.M.K. Singh**

*** University Dept. of Zoology, Ranchi University, Ranchi – 834001, Jharkhand, ** Central Tasar Research & Training Institute, Piska-Nagri, Ranchi – 835303, Jharkhand, India.**

ABSTRACT

Indian tasar silkworm, *Antheraea mylitta* D. has variegated scenario of its natural existence and in-vitro propagation. Different geographical pockets of the country, having deciduous forests are the natural abode of the silkworm which has been experiencing irrational anthropogenic interferences for centuries. Further, the wild silkworm has been subjected to multidimensional experimentation to enable it thrive successfully in semi-domesticated conditions and to produce more silk. The emergence of ecoraces in different natural habitats is, perhaps, the outcome of the various natural populations isolated geographically over centuries and result of adaptation to a particular niche, which has assumed the status of divergent ecoraces.

Present communication is an attempt to create cluster analyses followed by single linkage dendrogram from the *in-situ* cocoon production data on different host plants in different geo-eco pockets of tropical India. The information developed can be used to explore the degree of viability of certain ecoraces of divergent nature along with the trend of approximation of closely related ecoraces in a bid to promote composite commercial rearing rather than conventional ones and suggests an alternative option for providing seed crop at the time of scarcity.

INTRODUCTION

Silk and milk are two important consumables for the mankind produced by extreme phylogenetic groups of animals – mammals and insects. The unprecedented association of silk and man are equally inter-related to each other right from the Vedic period of affluency which was later on also supported by the historical silk route. Indian tropical tasar silkworm, *Antheraea mylitta* Drury is a wild polyphagous holometabolous lepidopteran saturniid insect with flexible voltine cycles. It thrives and propagates comfortably on naturally grown forest plants *viz.*, *Terminalia tomentosa* W&A (Asan), *T. arjuna* Bed (Arjun), *Shorea robusta* Gaertn. f. (Sal), and a number of secondary food plants available in deciduous forests of tropical India (Suryanarayana and Gangwar, 2007). Indian tropical tasar is mainly produced in the States of Jharkhand, Bihar, Chhattisgarh, Orissa, Madhya Pradesh, West Bengal, Andhra Pradesh, Maharashtra and fringes of Uttar Pradesh (Gangwar *et al*., 2007). During the journey of thousand years of its perpetuation, facing continuous depletion of the forests due to anthropogenic interferences (Stapanlan *et al*., 1997), the natural habitat of silkworm lost its continuity and geographically became isolated. This might be the causative factor for the development of ecoraces whose numerical abundance and qualitative ecorace richness related normally to each other in the habitat specific distribution which has never been looked into as the integral component of ecorace diversity and their viability index (Molles Jr., 2001).

In the present communication, the cluster analyses have been specially quantified in order to investigate the host and habitat specific propagation preference. The trend of approximation of closely related ecoraces to thrive successfully with little inter-ecorace competition and struggle over the available resources has also been explored (Stiling, 2002).

MATERIALS AND METHODS

Secondary data from published records (Suryanarayana and Srivastava, 2005) were compiled and analyzed using eco-statistical tools *viz.*, , Simpsion's similarity and Mantal's dendrogram, cluster analyses (Stiling, 2002). The statistical tools used were as follows:

Cluster Analysis: This procedure created one cluster from the 10 observations supplied. The clusters are groups of observations with similar characteristics. To form the clusters, the procedure began with each observation in a separate group. It then combined the two observations which were closest together to form a new group. After recomputing the distance between the groups, the two groups then closest together were combined. This process was repeated until only one group remained. To determine a reasonable value for the number of clusters, Agglomeration Distance Plot available from the list of Graphical Options were observed.

Apart from the above, **Box and Whisker graph** was also plotted to show the range of quantitative values of different parameters and their maximum periodicities taking all the ecoraces under observation (**Figure 1**).

RESULTS AND DISCUSSIONS

The published data (Suryanarayana and Srivastava, 2005) on the various attributes of 10 divergent ecoraces of *A. mylitta* D were considered in this work (**Table 1 & 2**). A unique picture of community similarity has been drawn by computation of **Cluster Analysis** by comparing their productive and commercial characteristics common to all the habitats of existence, as furnished in **Table-2**. Basically this exercise is an excellent measure of how many ecoraces are having similar attributes among the community (Stiling, 2002).

The overall result shown in **Figure 2 A&B**, based on productive and commercial characters, remarkably reveal that the Sal (*S. robusta*) fed ecoraces – 'Raily' and 'Modal', which are not amenable to human handling but have superior commercial characters, constitute a nearest neighbour cluster, along with 'Daba' and 'Sukinda'. Identically the commercially exploited ecoraces - 'Daba' and 'Sukinda', which are amenable to human handling, also form a close cluster based on commercial parameters. Based on commercial characters, 'Sarihan', 'Nalia' and 'Barharwa' are closer to each other; and 'Laria', 'Bhandara' and 'Andhra' are closer to each other. However, both the clusters of three ecoraces each have also close affinity to each other. An almost identical picture is revealed from **Figure 2.**

From the analysis, it is evident that two distinct clusters of divergent groups are formed based on the above parameters. The cluster 1 comprised of 'Raily', 'Modal', 'Daba' and 'Sukinda' while the second cluster comprised of 'Sarihan', 'Nalia', 'Barharwa', 'Laria', 'Bhandara' and 'Andhra'. All these ecoraces within the group are closer to each other while the races of the two clusters are quite apart from each other.

Due to polyphagous nature of the silkworm, the ecoraces thriving on different host plants have although developed divergent productive and commercial features significantly on the quality of the food resources nevertheless the cluster similarity in the

habitat has statistical governance controlled by other non-trophic factors of the environment.

The above results suggest three important options for rearing of Indian tropical tasar silkworm *viz.*, **(i)** The conventional concept of supplying of DFLs and rearing of silkworms of commercially exploited 'Daba' and 'Sukinda' ecoraces may be replaced by composite DFLs of either of the two close clusters mentioned above. This will not only check the in-breading depression in the lot, but will also create scope for improvement of the stock, **(ii)** The members of different clusters can also be utilized for race improvement by adopting and selecting suitable breeding methods, and **(iii)** The tasar growers will have an alternative option to rear other ecoraces of the said clusters rather than sticking on the traditional ones in the event of scarcity due to seed crop failure and cross infectivity of the diseases without sacrificing the quantity and quality of the cocoons.

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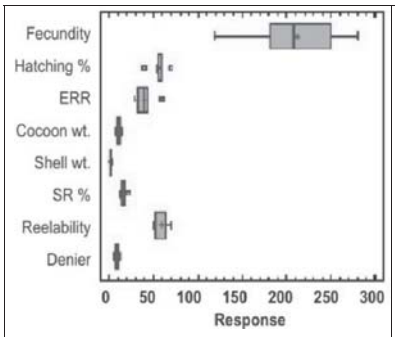

Figure 1: Box and Whisker plot showing different values (Range, Mean + Std. error) based on data presented in **Table 2**.

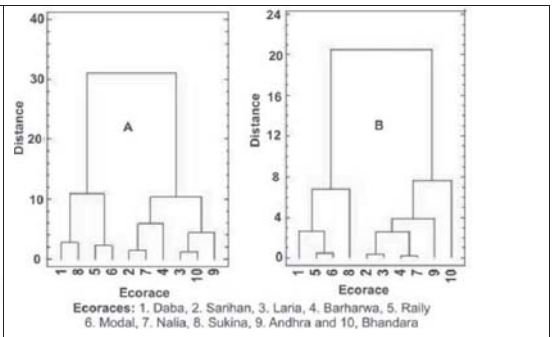

Figure 2: Dendrograms showing distinct clusters of selected ecoraces of *A. mylitta* D. based on **A.** Commercial characters (Fecundity, Hatching%, ERR) and **B.** Yield characters (Cocoon wt., Shell wt., S.R.%, Reelability, Denier)

Table 1: Characteristics of selected ecoraces and their habitats

Sl No.	Ecorace	Area of habitat	Geographical ordinates			Soil type	Forest type	Food Plant	Volti-nism	Mean max. T. (°C)	Mean min. T. (°C)	Ann. rain fall (mm)
			°N	°E	MA SL							
1	Daba	Singhbhum (Jharkhand)	22.1	86.2	209	Red loamy	Tropical moist deciduous	*Terminalia* spp.	BV	28.9	18.4	1095
2	Sarihan	Santhal Pargana (Jharkhand)	24.3	87.0	140	Red loamy	Tropical moist deciduous	*Terminalia* spp.	TV	31.54	20.52	1200
3	Laria	Peterbar (Jharkhand)	23.0	85.4	613	Red loamy	Tropical moist deciduous	*Terminalia* spp.	BV & TV	28.4	17.66	1100
4	Barharwa	Hazaribagh, Lohardaga (Jharkhand)	21.4	86.4	423	Sandy Red	Tropical moist deciduous	Terminalia spp.	BV	30.4	20.3	1218
5	Raily	Bastar (Chhattisgarh)	19.0	82.0	670	Red loamy	Tropical moist deciduous	*Shorea robusta*	UV, BV & TV	31.5	18.62	1276
6	Modal	Keonjhar (Orissa)	21.4	86.4	423	Sandy Red	Tropical moist deciduous	*Shorea robusta*	UV, BV & TV	30.4	20.3	1218
7	Nalia	Sundargarh (Orissa)	21.4	86.4	423	Sandy Red	Tropical moist deciduous	*Shorea robusta*	BV	30.4	20.3	1218
8	Sukinda	Sukindagarh (Orissa)	21.0	86.0	209	Red loamy	Tropical moist deciduous	*Terminalia* spp.	TV	31.45	20.6	1096
9	Andhra	Adilabad(Andhra Pradesh)	10.4	79.1	170	Black claye y	Tropical dry deciduous	*Terminalia* spp.	TV	31.52	21.8	925
10	Bhandara	Bhandara (Maharashtra)	21.0	79.4	311	Black claye y	Tropical dry deciduous	*Terminalia* spp.	TV	34.5	20.8	939

Table 2: Productive and Commercial characters of various Ecoraces of *A. mylitta* D.

Sl. No.	Ecorace	Fecundity	Hatching (%)	ERR (%)	Cocoon Wt (gm)	Shell Wt. (gm)	SR % (%)	Reelability (%)	Denier
1	Daba	250	70	40	14.00	2.00	16.8	56.7	10.0
2	Sarihan	201	56	42	9.75	0.85	13.0	52.0	9.0
3	Laria	215	55	37	7.60	1.40	17.1	61.0	8.0
4	Barharwa	166	54	31	10.00	1.70	17.0	50.0	8.0
5	Raily	280	60	42	12.65	2.85	18.4	62.7	10.0
6	Modal	249	58	40	14.00	3.10	22.0	65.4	10.0
7	Nalia	182	55	28	6.50	0.90	13.0	52.0	8.0
8	Sukinda	266	60	60	12.00	1.60	13.5	65.0	9.0
9	Andhra	119	55	41	7.60	1.10	14.0	69.0	8.0
10	Bhandara	191	40	30	8.40	1.32	16.0	63.0	8.0

Impact of Human Resources Development on Tropical Tasar Silk production in India.

S.K. Gangwar*, V.P Gupta, S.N. Sinhadeo, N.Suryanarayana and N.B. Vijaya Prakash. Central Tasar Research & Training institute, Piska-Nagri, Ranchi – 835 303, Jharkhand, INDIA**

ABSTRACT

In a bid to find out the possible impact of human resources development on tropical Tasar silk production in India, data for past 55 years (1971-2005) on raw silk production and various types of trainings imparted by Central Tasar Research & Training Institute (CTR&TI), Ranchi (INDIA) were collected from secondary sources and analyzed on five yearly pooled basis. The results revealed that there was a steep increase in Tasar raw silk production after 1966 with peak production during second half of 1990s (463.60 MT/year), but the same started declining thereafter bringing it below 300 MT/year. Peak raw silk production coincided with mass training programmes organized by CTR&TI, Ranchi for candidates sponsored by Tasar producing States. Training programmes of comparatively longer duration showed stronger positive correlation with Tasar raw silk production. Relationship between Tasar raw silk production and Post Graduate Diploma in Non-mulberry Sericulture (PGDS-NM) of 15 months duration was significant at 5% level.

INTRODUCTION

Human resources development (HRD) is known to be the backbone of an organization. Sound training system in an organization not only keeps the staff updated in contemporary knowledge, but also helps it fulfilling its goals efficiently and effectively. Silk production being skilled avocation, trainings find special sheet of importance. Since silk production involves a chain of activities handled and supported by different people of varying skills and operation areas, it is rather difficult to assess the impact of different types of trainings on silk production. Mainly four types of people are involved in the silk production *viz.*, 1) Innovators (Scientists), Supporters (Technical and other supporting staff, 3) Transducers (Extension workers) and 4) End users (Farmers). These people have different levels of qualifications and skills, and therefore, need different types of trainings. Innovators develop new materials/ technologies with the help of supporting staff. Transducers take the new materials/ technologies to the end users who in turn use them for their benefits.

Tropical Tasar in India is confined to nine States *viz.,* Bihar, Jharkhand, Chhattisgarh, Madhya Pradesh, Orissa, West Bengal, Andhra Pradesh, Maharashtra and Uttar Pradesh. Jharkhand and Chhattisgarh were carved out of Bihar and Madhya Pradesh respectively during the year 2000 and hence their old records are available in the combined form of parental States. Central Tasar Research & Training Institute CTR&TI), Ranchi is the nodal agency under Central Silk Board, Govt. of India responsible for R&D activities and for supporting State sericulture departments in the development of Tasar silk. It was established in the year 1964 and, since then, has devised various types of training programmes for different groups of people involved for the development and production of Tasar silk. These training programmes are designed to suit the need of the Tasar silk industry and implemented time to time since 1969. Since then a large number of candidates have been trained in various types of trainings and no attempt has so far been made to assess their impact on the Tasar silk development. However, in mulberry sericulture, efforts have been made to evaluate

knowledge and adoption level amongst trained farmers (Srinivas *et al*., 2007). It was, therefore, decided to find some easy ways to assess the same.

MATERIAL AND METHODS

State-wise and yearly data on Tasar raw silk production since 1951 were collected from Statistical Biennial and Annual Reports of Central Silk Board, Bangalore (India). During the year 2000, three new States were carved out of the old States *viz*. Jharkhand from Bihar, Chhattisgarh from Madhya Pradesh and Uttaranchal from Uttar Pradesh. Hence, for uniformity, combined data for these pairs of States were used for the period beyond the year 2000. Data on different types of trainings imparted at Central Tasar Research & Training institute (CTR&TI), Ranchi (India) were collected from official records where the authors are working and compiled. Training programmes conducted at CTR&TI varied in their types and duration, and this posed a problem of their uniformity for comparison with raw silk production during corresponding periods. Further, there was no uniformity in types and duration of training during the entire period of study (1951 to 2005). It was, therefore, decided to group the training programmes into uniform categories depending upon their types, duration, target groups *etc*. One more approach, to bring the data closer to uniformity, was thought to pool the data for a considerably longer duration. Thus the data for Tasar raw silk production were averaged for over five yearly intervals, while the same for number of candidates trained under different training courses were summed for the corresponding five yearly bases.

Since graphical representation is easily understood when comparisons are made for more than one variable, data on State-wise Tasar raw silk production were plotted against five yearly periods. Similarly, data on major identical training programmes *viz.,* Post Graduate Diploma in Non-mulberry Sericulture (PGDS-NM) of 15 months duration, Refresher Training Course (RTC) of 14-60 days, Special Training Programme for Graduate candidates (STP(G)) of Six months, STP(M) = Special Training Programme for Matric (X standard) pass candidates (STP(M)) of Six months, Special Short-term Training Course (SSTC) of 15-30 days, and Farmers Training Programme (FTP) of 60 days were pooled for corresponding periods. However, State-wise data on all the training programmes were not available except PGDS-NM and RTC and thus correlation analysis could be made on the data pooled over the different States, and plotted along with the common graph of Tasar raw silk production and candidates trained under different types of trainings.

RESULTS AND DISCUSION

Data presented in **Figure 1A** indicate that the bulk Tasar raw silk is produced in the States of Bihar/Jharkhand, Madhya Pradesh/ Chhattisgarh and Orissa with West Bengal being other State contributing considerably to this commodity. Of late, Andhra Pradesh has also started considerable production of Tasar raw silk and during the period 2001-2005, its annual production reached to 14 MT. Maharashtra and Uttar Pradesh/Uttaranchal are somehow surviving on the map of Tasar raw silk production with their annual production ranging between 1-6 MT.

Pooled data over the States indicate that there was sharp increase in raw silk production during the second half of the 1970s, and its peak is visible during the second half of 1990s with average annual production reaching to 463.60 MT during 1986-90. After this period, the production of raw silk started declining and average annual production went down to 247.40 MT/year during the period 1996-2000. This trend was

also visible in most of the States except Madhya Pradesh where peak raw production of 149.60 MT/year was recorded during the period 1966-70.

The data presented in **Figure 1B** indicate that West Bengal, Madhya Pradesh, Andhra Pradesh and Bihar sponsored bulk candidates for 15 months PGDS-NM course during the period from 1976 to 1990. This period coincided with the peak production of Tasar raw silk. Candidates sponsored by different States for RTC of shorter duration 14-60 days had no specific trend (**Figure 1C**). However, during the period from 1970 to 1990, there was a massive special training of 6 months duration for two separate classes of people *viz.,* STP(G) for graduate and STP(M) Matric pass to suit the syllabus of their palatability. Similarly, there was a massive Stipendiary FTP for farmers during the corresponding period as indicated in **Figure 1D.**

Date presented **Figure 1E** indicate that both the Tasar raw silk production and long duration training programmes (PGDS-NM, STP(G), STP(M)) had their peaks during similar periods and hence indicated their positive relationship which was reflected in correlation coefficient (**Figure 1E**). However, only PGDS-NM course showed significant correlation at 5% level. It is worth mentioning that the long duration programmes like PGDS-NM and STP during their peak activities were due to sponsoring of either in-service candidates or need-based candidates to be absorbed by the concerned States in their State level sericulture departments. Proper utilization of their services after training might be the possible reason for increased production of Singhvi *et al*. (1994) have reported that lack of awareness in one of the reasons for non-adoption or partial adoption of the new technologies by farmers thereby resulting in low cocoon yield. Tasar raw silk during the corresponding periods. Short-tem courses were mostly used for in-service candidates of Central Silk Board or the candidates sponsored by non-government organizations with no proper use of their services in developmental works after training.

It is therefore clear from the present study that the training, particularly, long term training with clear cut goal and post training engagement of these trained hands has a positive impact of the growth of Tasar industry in the country. The study also opened a platform for discussion of chalking out suitable HRD programmes for different classes of people and proper engagement of the trained manpower for the benefit of Tasar silk industry in the country.

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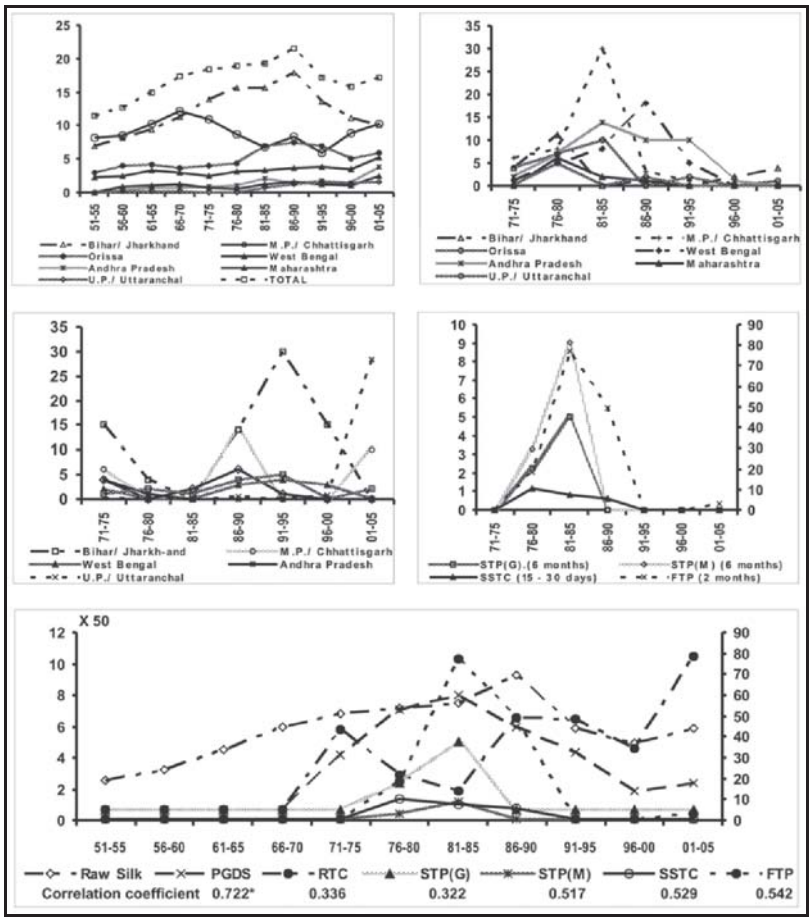

Fig 1: Tasar Raw silk production and different trainings imparted in selected States of India during past 55 years (Data average of five yearly intervals)

Note: Raw silk MT/year, Trainings (No. of candidates trained): PGDS = Post graduate Diploma in Non-mulberry Sericulture (Duration 15 Month), RTC = Refresher Training Course (14 – 60 days), STP(G) = Special Training Programme for Graduate candidates (Six months), STP(M) = Special Training Programme for Matric pass candidates (Six months), SSTC = Special Short-term Training Course (15-30 days), and FTP = Farmers Training Programme (60 days)
- All data pooled over five yearly intervals

Variability, Heritability and Correlation of Quantitative Traits in Castor (Ricinus communis L.).

S.N. Gogoi, Meghali Barua and R. Chakravorty. Central Muga Eri Research & Training Institute, Central Silk Board, Lahdoigarh, Jorhat–785700, Assam, India.

ABSTRACT

An experiment was conducted with 72 castor genotypes to determine the genetic variability, heritability, genetic advance and correlation of 10 quantitative traits. In general phenotypic coefficient of variation (%) were found to be higher than genotypic coefficient of variation (%). Genotype contributed more than environment in the expression of plant height, spike length and width, number of fruits/spike, leaf moisture and leaf yield/plant. Plant height, spike length and width, number of fruits/spike, leaf moisture and leaf yield/plant showed high broad sense heritability along with high genetic advance (GA) as percent of mean indicating presence of good amount of additive genetic components. 100 seed weight was found to be highly influenced by environment as it exhibited low heritability with low genetic advance and selection based on this would be ineffective. Correlation coefficient indicated positive relation of leaf yield with number of branches, leaf area and 100 seed weight.

Key words: Castor, genetic variability, heritability, genetic advance, correlation, quantitative traits.

INTRODUCTION

Evaluation of castor gene pool for important agronomic traits is prerequisite for castor improvement. A breeder is always concern with the selection of superior genotype which perforce is dependent on phenotypic expression. Heritability and genetic advance are important selection parameters which help in selection of elite genotypes from diverse genetic populations. Often selection based on phenotypic performance does not lead to expected genetic advance mainly due to genotype (g) x environmental (e) interaction. Thus knowledge of complex characters like yield and its component characters help to select superior genotypes. The efficiency of selection mainly depends on the extent of genetic variability and the heritability of the concerned characters. The phenotypic variability is due to genetic and environmental causes, of which only the former one is heritable. The heritable portion of the variation was determined by heritability estimates. Johnson *et al*. (1955) suggested that heritability estimates along with genetic advance is usually more helpful than the heritability value alone in predicting the resultant effect of selecting the best genotype. Knowledge of correlations between different characters is useful for selection of traits having low heritability which cannot be selected directly. Such traits can be improved by selecting associated traits. According to Dogra (1981) selection of superior phenotypes for improvement programme should be based on local species rather than exotics because these species are better adapted for survival and productivity as well

Castor *(Ricinus communis L)* is economically important as a primary food plant of eri silkworm, *Samia ricinus* (Donovan) and as oil seed crop in India. India has monopoly in

the international castor oil trade (Damodaram and Hagde, 2005). Castor shows wide range of variability both in natural habitats (Gogoi *et al*. 2005) and wild and semi wild habitats (Anjani, 2005). As an oil seed crop, seed yield is an important trait and as a host plant of eri silkworm, leaf yield is an important trait.

Therefore, an attempt has been made to study genetic variability, heritability, genetic advance (GA) and correlation of various yield and its component characteristics in the castor germplasm of Northeast India to determine criteria for selection of promising castor genotypes for improvement of eri cocoon production.

MATERIALS AND METHODS

Seventy two castor accessions were collected from different parts of Northeast India which lies between $24^0$8'-$29^0$28' N latitude and 90^0- 97^0 E longitudes, were used for study. NBR1 (Accession1) was taken as check. Seeds of each accession were sown in experimental field at Central Muga Eri Research and Training Institute (CMER&TI), Lahdoigarh, Jorhat, Assam, India for preliminary yield trials following recommended package of practices. Each experiment was laid out in RBD with 3 replications in 2 rows plot of 10m length and 2m width. A spacing of 1x1 m between plants was maintained. Ten random plants were selected from each plot for recording observations on 10 quantitative characters viz., plant height, number of branches, internode length, leaf area, spike length and width, number of fruits per spike, 100 seed weight, leaf moisture content and leaf yield per plant. Analysis of plot means data were done for genetical parameters viz., phenotypic and genotypic co-efficient of variation, broad sense heritability and genetic advance following standard statistical procedures (Lush, 1949; Johnson *et al.*, 1955 and Searle, 1961).

RESULTS AND DISCUSSIONS

The mean values of the characters recorded during preliminary yield trials of all 72 castor accessions are presented in Table 1. The mean table revealed A48 to be tallest (230.67cm) and A26 to be shortest (115.33cm). Four accessions viz., A31, A34, A44 and A52 had only one branch but A56 had the maximum (6.3) with leaf yield of 3.28 kg/plant indicating as promising accession for leaf yield. Internode length was maximum in A25 (12.32cm) and minimum in A38 (4.5cm). Highest leaf area (2634.34cm^2), leaf yield/plant (4.45kg), spike length (55.28cm), number of fruits/ spike (83.00) and 100 seed weight (29.3g) were observed in A4 indicating it as most promising accession for both leaf and seed yield. Lowest leaf area (314.17cm^2) and leaf yield per plant (0.43kg) were found in A33. Lowest spike length was of A32 and A68 (17.25cm). A67 produced lowest number of fruits per inflorescence (12.31) and A6 produced seed of lowest weight (8.37g/100 seed). Highest spike width was seen in A68 (27.63cm) and moisture percent in A22 (78.66%). A23 produced spike of lowest width (6.5cm) and leaf with lowest moisture content (50.78%).

Variance components of all the characters assessed were shown in Table-2. For better comparison all the values were taken in percent. From the table, highest phenotypic coefficients of variation (PCV %) was observed in leaf area (67.96) and highest genotypic coefficients of variation (GCV %) was observed for leaf yield/plant (54.4%). In general the PCV were higher than GCV but the difference between them found to be narrow for plant height, spike length and width, number of fruits/spike, leaf moisture and leaf yield/plant implying that genotype contributed more than environment in the expression of these characters and hence selection based on phenotypic values is possible. However, number of branches, internode length and leaf area showed

comparatively wider difference between PCV and GCV implying larger influence of environment on these characters.

The heritability estimates in percentage range from 18.94% to 88.28%. High heritability estimate (>70%) were seen in plant height, spike length and width, number of fruits/spike, leaf moisture and leaf yield/plant. Moderate heritability (>50% to <70%) was shown by number of branches per plant and internode length. Low heritability (<50%) was recorded for leaf area and 100 seed weight. The expected genetic advance with selection intensity at 5% level (k=2.06) was calculated as percent of mean which varied from 8.97% (100 seed weight) to 105.13% (leaf yield/plant). Plant height, spike length and width, number of fruits/spike, leaf moisture and leaf yield/plant showed high broad sense heritability along with high genetic advance (GA) as percent of mean indicating that these traits are under strong genetic control and have good amount of additive genetic components. Thus, there is ample scope for improving these traits based on direct selection. High heritability coupled with moderate GA indicates the presence of additive gene effects (Panse, 1957). Solanki *et al.* (2003) reported significant contribution of additive gene effects on number of fruits/spike, number of fruits and primary spike length. Number of branches and internode length showed moderate heritability with moderate genetic advance as percent of mean confirming the role of both additive and non additive gene action. 100 seed weight showed low heritability with low GA indicating the role of non additive gene action. In both the case, there is limited scope of improving the traits by selection.

Results of correlation coefficient between different characters are presented in Table-3 which revealed high degree of association between few traits, indicating that a limited numbers of characters could be considered in variety development programme of castor for eri silkworm rearing. Leaf yield, number of fruits per spike and 100 seed weight are economically most important characters and correlation coefficient analysis revealed positive relation of leaf yield with number of branches, leaf area and 100 seed weight. Banerjee *et al*. (2007) concluded leaf area as an important component for leaf yield improvement in mulberry whereas Ramu *et al.* (2005) indicated significant positive association of seed yield with oil yield. Thus it is possible that plant selected for higher leaf yield will also give high seed yield. Number of fruits per spike positively related with plant height, leaf area, spike length and width. Similar results were reported in castor under dry land condition (Kumar *et al*., 2003). 100 seed weight was related with leaf area, leaf yield and moisture percent of leaf.

The degree of success in castor improvement programme depends on the genetic variability in the genotypes and their utilization. The estimate of genetic parameters like genotypic coefficient of variation, heritability and genetic advance are needed to formulate suitable breeding procedures and to foresee the possibilities up to which extent a particular character could be improved.

From these analysis we can confirmed the presence of sufficient variability in the collected castor germplasm. The characters viz., leaf yield, fruits per spike, 100 seed weight, and number of branches, leaf area, plant height, spike length and width appears to be of high importance and based on these characters indirect selection is likely to be more effective in genetic improvement programme of this species in future. Thus castor genotypes available in this region can be developed for both purpose i.e. leaf yield and seed yield.

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Table 1. Performance of castor genotypes under preliminary yield trials

Accessions	Plant height (cm)	No. of branches	Internode length (cm)	Leaf area (cm²)	Spike length (cm)	Spike width (cm)	Fruit per spike	100 seed wt. (g)	Leaf moisture (%)	Leaf yield/plant (kg)
A1(NBR 1)	150.67	3.6	8.50	793.35	45.26	10.83	43.64	14.15	62.31	2.30
A2	162.67	4.0	7.83	1494.62	45.64	10.53	51.38	15.25	67.98	0.90
A3	220.83	4.0	8.53	1547.60	45.00	10.80	51.45	15.88	66.32	3.85
A4	201.27	3.6	7.61	2634.34	55.28	10.78	83.00	29.30	77.51	4.45
A5	184.61	2.8	7.05	750.87	37.67	12.11	39.55	12.95	65.06	1.09
A6	171.24	3.3	7.34	650.33	28.40	10.83	25.40	8.37	66.11	0.46
A7	183.62	2.1	8.50	1267.34	40.33	10.17	40.83	17.41	64.75	0.82
A8	174.33	2.6	8.50	666.65	40.25	9.47	37.30	14.78	63.23	0.74
A9	177.65	2.1	7.86	849.37	31.33	8.33	32.67	13.92	68.28	0.79
A10	184.00	2.6	9.11	1190.00	30.10	10.60	32.66	13.97	65.98	2.06
A11	213.72	5.6	10.07	817.26	36.83	10.93	32.07	16.10	74.81	3.15
A12	185.31	3.5	7.83	1240.50	36.48	10.43	32.56	15.65	62.44	1.35
A13	187.45	4.0	8.55	1098.30	34.66	11.27	37.12	13.15	66.38	2.25
A14	197.74	3.5	7.80	984.48	44.50	10.03	48.33	15.00	65.43	0.83
A15	181.00	3.3	7.34	940.59	38.50	10.63	44.00	13.02	68.02	1.21
A16	163.61	3.3	7.86	1298.00	46.37	10.20	50.60	12.81	70.14	1.34
A17	145.84	3.8	7.50	750.67	34.20	10.75	44.35	16.74	69.69	1.39
A18	155.66	3.6	8.17	962.35	34.46	11.24	43.64	12.41	68.09	1.26
A19	137.33	2.3	6.50	1547.33	34.43	10.77	43.62	15.62	73.32	2.20
A20	213.41	3.8	7.74	1349.00	33.53	10.93	44.42	14.52	74.92	1.33
A21	164.75	4.5	7.39	821.17	45.00	10.83	41.85	24.88	72.45	2.45
A22	200.99	5.0	7.25	2505.32	55.08	10.00	65.55	25.74	78.86	4.28
A23	188.00	3.5	10.15	1556.14	19.42	6.50	23.23	17.39	50.78	3.34
A24	165.43	3.1	5.40	588.67	41.09	9.08	20.67	14.97	71.39	1.41
A25	165.67	4.0	12.32	1199.67	25.44	10.67	30.33	16.43	72.77	0.76
A26	115.33	2.6	11.20	725.44	30.00	10.13	27.33	14.05	69.56	2.48
A27	190.44	2.3	6.63	717.59	32.74	10.83	38.00	13.09	75.17	0.57
A28	204.00	2.8	6.50	713.58	21.67	6.67	20.09	15.84	76.76	2.34
A29	134.21	2.5	7.04	1178.83	23.00	8.50	38.05	18.81	71.51	1.45
A30	177.62	2.0	8.83	1662.80	28.30	14.57	55.28	25.84	77.02	4.35
A31	141.23	1.0	6.34	793.50	39.05	9.17	45.06	18.64	68.92	2.41
A32	175.53	3.6	8.65	503.50	17.25	8.27	30.44	15.68	67.85	2.29
A33	139.24	2.1	7.44	314.17	32.00	11.03	54.25	14.42	64.17	0.43
A34	164.00	1.0	5.64	770.55	32.55	12.00	52.15	11.80	61.76	0.49
A35	193.62	2.6	10.30	1003.73	25.17	9.66	32.64	14.59	64.97	2.42
A36	173.00	4.0	6.53	525.67	21.67	9.50	20.34	21.96	67.01	3.68
A37	167.57	4.3	7.65	503.83	27.62	11.48	31.14	14.77	67.01	0.82
A38	135.53	1.1	4.50	405.70	29.56	10.45	33.73	13.05	62.93	1.59
A39	170.45	1.3	6.54	749.87	31.62	10.30	55.62	16.91	68.29	0.72
A40	169.22	3.6	10.09	1792.23	42.33	14.05	64.67	14.91	73.06	0.77
A41	166.15	2.8	7.80	1263.47	26.08	10.84	28.30	26.54	75.46	1.24
A42	188.21	2.0	6.67	542.67	33.47	11.60	64.60	14.16	67.35	0.98
A43	186.65	2.3	8.50	878.50	31.55	9.83	48.41	16.19	76.70	0.61
A44	167.67	1.0	9.74	748.54	28.60	8.88	22.69	14.97	67.68	2.57
A45	188.82	2.5	5.81	375.00	42.36	11.53	74.58	15.81	68.33	2.46
A46	161.34	3.1	8.68	1054.35	25.90	10.10	33.64	11.96	67.21	2.10
A47	231.64	5.0	5.84	682.37	30.07	9.77	41.35	14.17	67.21	2.14
A48	230.67	3.0	6.67	1663.47	26.81	9.47	28.41	16.39	65.52	3.37

A49	188.7	2.6	6.60	517.30	22.32	6.65	19.60	14.66	75.00	1.90
A50	134.29	2.3	7.17	431.32	29.09	15.32	50.64	15.57	76.72	2.28
A51	167.46	2.0	7.83	707.00	27.44	11.10	47.34	17.64	75.03	2.77
A52	138.00	1.0	6.47	796.19	38.68	8.67	47.05	11.09	75.53	2.20
A53	188.54	3.3	7.53	883.67	20.09	8.75	27.66	11.80	75.92	1.98
A54	176.20	2.8	6.50	718.00	31.57	11.54	42.37	15.56	70.87	2.81
A55	168.33	3.6	6.83	507.00	36.60	11.26	42.65	15.81	76.63	1.31
A56	197.30	6.3	10.27	699.66	47.66	11.37	35.62	15.60	72.79	3.28
A57	189.00	4.0	7.32	1334.83	25.34	9.68	24.58	15.88	76.47	2.52
A58	161.00	3.1	6.33	810.45	34.38	10.33	49.34	16.63	70.95	2.59
A59	206.66	4.0	9.47	932.62	51.34	15.00	75.64	12.00	66.46	1.22
A60	184.87	4.1	7.60	562.00	49.06	13.30	64.51	13.84	71.38	1.65
A61	155.00	3.6	6.00	836.11	24.48	9.83	29.22	16.86	71.55	1.04
A62	138.64	1.8	7.88	1651.77	35.75	10.50	31.30	14.01	68.01	2.16
A63	161.25	3.1	8.83	1633.30	47.62	13.44	70.08	15.62	67.04	2.06
A64	186.38	3.3	10.67	735.55	33.58	17.21	79.00	15.71	72.90	1.16
A65	180.65	3.0	8.17	1434.35	36.46	11.58	48.92	16.93	71.64	0.73
A66	153.72	3.3	7.75	628.00	24.66	9.32	35.14	19.87	71.61	0.74
A67	150.80	3.1	3.28	656.72	37.28	13.52	12.31	15.60	73.98	1.88
A68	200.36	4.0	5.68	685.80	17.25	27.63	35.72	15.69	61.76	2.34
A69	150.70	4.0	8.40	1160.50	22.53	9.54	27.16	14.85	60.18	1.92
A70	203.33	3.1	7.30	1156.61	29.25	9.08	35.55	15.41	70.91	2.61
A71	203.33	3.3	7.61	1424.42	29.54	9.52	35.25	12.65	68.36	0.95
A72	174.67	3.3	8.50	651.44	35.40	8.17	39.04	13.56	66.77	1.01
CD-5%	26.94	1.45	1.87	973.08	7.51	1.77	8.25	8.24	1.78	0.69

Table 2. Estimates of genetic parameters for different characters of castor accessions.

Characters	Co-efficient of variation (%)		Heritability (broad sense)	Genetic advance (k=2.06)	Genetic advance as % of mean
	Phenotypic	Genotypic			
Height of plant (cm)	23.08	20.94	82.28	66.59	39.12
Number of branches	41.49	29.35	50.03	1.31	42.76
Internode length (cm)	26.88	22.12	67.70	2.81	37.49
Leaf area (cm^2)	67.96	29.57	18.94	261.83	26.51
Spike length (cm)	27.44	24.39	79.00	15.07	44.66
Spike width (cm)	19.59	16.70	72.67	3.11	29.33
No. of fruits/spike	37.66	35.29	87.78	28.2	68.10
100 seed wt. (g)	9.97	6.59	43.66	6.14	8.97
Leaf moisture (%)	21.70	21.08	94.37	6.63	42.19
Leaf yield/plant (kg)	57.98	54.4	88.02	1.98	105.13

Table 3. Correlation coefficient among various quantitative characters of castor accessions collected from various parts of Northeast India.

Characters	Plant height	No. of branch	Internode length	Leaf area	Spike length	Spike width	Fruits/ spike	Leaf moisture	100 seed wt.	Leaf yield
Plant height		.135*	.282**	.159*	.066	-.093	.183*	.025	.029	.118
No. of branch			.147*	.141*	.178*	.101	-.044	.106	.077	.135*
Internode length	.			.017	.045	.161*	.098	-.015	.086	.014
Leaf area					.241**	.055	.209**	.093	.239**	.281**
Spike length						.413**	.576**	.029	.077	.053
Spike width							.564**	.077	.028	-.031
Fruits/ spike								.101	.104	.047
Leaf moisture									.175**	.119
100 Seed wt.										.407**
Leaf yield										

** Significant at the 0.01 level.
* Significant at the 0.05 level.

Biodiversity of Eri silk worm and their molecular characterization.

R. Chakravorty[1], K. C. Singh[2], K. Neog[1] , B. B. Singha[3], , B. N. Sarkar[1], S.A.S. Rahman[1], Pranab Dutta[1], H.J. Anuradha[4], N. Rawat[4], A.R. Pradeep[4], C.V. Nair[4], A.K. Awasthe[4],S. Raje Urs [4] .
[1]Central Muga Eri Research and training Institute, Lahdoigarh, Jorhat-785 700, Assam, India, [2] Regional Tasar Research Station, Central Tasar Research and Training Institute, Imphal, India, [3] Regional Eri Research Station, Mendipathar, East Garo Hills, Meghalya-794 4-112, India,
[4]Seribiotech Research Laboratory, Central Silk Board, CSB campus, Kodathi, Carmelram, P.O., Bangalore-560 035, Karnataka, India.

Abstract:

Eri silk worm, *Samia ricini* contributes significantly to the Indian commercial Silk production which is widely distributed in the Brahmaputra river valley in North Eastern region of India. As over exploitation with rapid natural calamities, deforestation etc. causing dwindling of natural population of *S. ricini* therefore, all the eri silkworm germs of the region in need to explore, collect and enrich the germplasm bank with silkworm genetic materials with high variability based on distinct morphological and quantitative traits in order to conserve them for future utilization in breeding programmes. Twenty six collections made on entire North East were assigned to accession number and were characterized based on heritable morphological character. Different heritable characters e.g., large black mottles on the integument, blue (greenish blue) vs. white (or yellow) integument and the white and brick red cocoons were recorded in different accessions. Ten accessions' viz., 001, 002, 003, 004, 005, 006, 011, 015, 018, and 025 were found as productive on the basis of morphological variability present within and between eco-races in terms of evaluating characters. Molecular study on heterzygocity showed a total heterozygosity of 0.2957±0.0363 and 0.2934±0.0420, respectively and Nei's gene diversity as 0.2957±0.19404 and 0.2934±0.2050, respectively for SRI-006 and SRI-003 population. Study on the DNA fingerprinting using the six eco-races (001 to 006) showed that gene flow between the populations of 003 and 005 is quite high (0.9035) and lowest (0.2172) is between 002 and 003, but have similar in phenotypic traits, such as cocoon colour. The genetic distance is lowest between 003 and 005 (0.0654) and highest between 002 and 006 (0.3811).

Key Words: Eri silkworm, land races, morphology, heterozygosity,

Introduction:

Eri silkworm, *Samia ricini* (Donovan) is a domesticated non-mulberry, polyphagous, multivoltine silkworm reared on the leaves of castor (*Ricinus communis*) and kesseru (*Heteropanax fragrans*). The insect has been exploited commercially for its silk since time immemorial. The silk produced by eri silkworm is considered economically the third most important silk in the world after mulberry silk and Chinese tasar. Eri culture enjoys a unique position among other sericulture activities like Mulberry, Tasar and Muga for its typical quality of white soft yarn with thermal property. North East Region of India is a natural abode for distinctly diversified wide range of flora and fauna as the entire four silkworm have been practiced and sericulture as a whole has a close link with the culture and tradition of the people of the region. The Brahmaputra valley of Assam and its adjoining food hills in the Sub-Himalayan belt is believed to be the native of eri.

Two species of eri silkworms available in North- East India, viz., the domesticated *S. ricini* and its wild progenitor *S. canningi* (Hutton). Depending on availability of food plants and prevalence of favourable climatic conditions, 6 crops of eri can be raised in a year and is widely practiced among 1,49,466 families in this region contributing about 73% of the total raw silk produced in India. About 7,793 hectares of land is available under eri host plants in the northeastern states of India. Over the period of last twenty years, eri silk production increased significantly from 279 MT in 1984-85 to 974 MT in 1999-2000, further to 1448 MT during 2004-05. India produced about 1448.20 MTs of eri raw silk per year surpassing production of tasar silk (322 MTs) and muga silk (110 MTs).

Although, eri silkworm rearing is practiced in NE India from time immemorial yet the details of the races reared are not available. In general, all the eri silkworm accessions of the NE India are in need to explore, collect and enrich the germplasm bank with silkworm genetic materials with high variability based on distinct morphological and quantitative traits in order to conserve them for future utilization in breeding programmes.

With this background investigations were carried out since 2003 to till date with an aim to explore eri silkworm germplsm resources of the NE region with the objectives, to collect endangered varieties / land races of eri silkworm, genetic variability and molecular characterization.

Materials and Methods

The present study was carried out in the Eastern Himalayan Region of Indian continent (22° - 29° 30´N, 80° 05´ - 97° 24´S and 97 to 5000 m above MSL) with three river basins: the Brahmaputra, Barak and Tista, and eastern Himalayan range along with peninsular plateaus of Meghalaya and Karbi Anglong. The area is with an average annual temperature of 20°C, maximum and minimum being 34.6°C and 19.9°C respectively in August and 23.9 and 7.1°C in January and receives annual precipitation as 2,809 mm. The climate best fits the zone of heavy rainfall with pronounced dryness by the winter months of December and February.

Passport data, required for taxonomic study, and documentation of the germplasm were collected at the time of silkworm germplasm collection on five different parameters viz., race name, donor, origin, class, parentage and were assigned a accession no. Embryonic and post embryonic characteristic for all the accessions were studied and analyzed statistically.

Characterization of eri silkworms was done based on heritable morphological character of *S. ricini* which were recorded during the different stages of growth period as per descriptor (Chakravorty, *et al*., 2004b).

To estimate the genetic variability within and between the eco-races of *S. ricini* based on fingerprinting with SSR and ISSR and degenerate primers, 15 eco-races of *S. ricini* collected from different parts of N. E. India were analyzed for molelecular characterization by Seribiotech Research Laboratory (SBRL), Kodathi. The DNA was extracted from 5th instar silkworm larvae following the method of Thanananta et al. 1997; Nagaraja & Nagaraju 1995.

The eri individuals of SRI-009 populations were analyzed using 840 ISSR and 885 ISSR primer. SRI-001, SRI-003 and SRI-007 population were analyzed by 886

ISSR primer and ISSR primer 811 was used for analyzing the individuals of SRI-002 population. PCR based molecular marker, random amplified polymorphic DNA (RAPD) technique was also used to study the DNA profiles of the eri silkworm populations.

Results and discussion

Passport data required mainly for taxonomic study and documentation of eri germplasm were collected at the time of silkworm germplasm collection presented in Table 1 showed a collection of 26 accessions with different race name and origin though their parentage were same. These were assigned to unique accession number as SRI-001, SRI-002 and so on. The accession number prefixed by SRI stands for *S. ricini* and suffixed by 001, 002 means serial number of collection for each race name.

Heritable characters e.g., large black mottles on the integument, blue (greenish blue) vs. white (or yellow) integument and the white and brick red cocoons were also recorded and listed in the Table 2 for the purpose of documentation. Data recorded on embryonic and post embryonic stages of eri silkworm, viz., egg, larva, cocoon / pupa and adult stage based on the descriptors. Data presented in Table 2 showed morphological variability in all the studied characters viz., fecundity (%), hatching (%), larval wt (g), larval period (days), effective rate of rearing (ERR) (%), cocoon wt. (g), shell wt (g) and shell ratio. Fecundity of the collected germplasm varies from 318.50 to 496.41 for SRI-012 and SRI-018 respectively. Hatching per cent of all the germplams were found different from each other being highest (95.04%) for SRI-001 with a lowest of (71.20 %) for SRI-009(Fig 2). No significant variation in larval weight and larval period were recorded for the population. ERR was found different in accordance with the populations. Maximum ERR (92.00 %) was recorded for the population SRI-026 followed by SRI-002 (90.52 %), SRI-020(90.52 %), SRI-012 (90.50 %) and SRI-005 (89.25 %)(Fig 2) without much different statistically. Data recorded the cocoon weight for the collected germplasm were found statistically at par with each other. On the other hand maximum shell weight (g) was recorded for the accession SRI-018 followed by SRI-001, SRI-021, SRI-002, SRI-003, SRI-005, SRI-006 SRI-003 and so on. Though the population SRI-025 showed shell weight much lower than the populations viz., SRI-018, SRI-001,SRI-021, SRI-002, SRI-003, SRI-005, SRI-006 SRI-003(Fig 1)but it showed highest shell ratio(14.59%) as compared to all the accessions studied. Shell ratio of the accessions SRI-001, SRI-021, SRI-003 and SRI-006 with 13.74%, 13.54%, 13.52% and 13.20% respectively were next to SRI-025 (Table 2).

The morphological variability present within and between eco-races in terms of evaluating characters was statistically analyzed on each descriptor with continuous variation to determine the means, minimum and maximum values, variance, standard error, skewness, and kurtosis (Table 3) and found that out of the total 26 *S. ricini* accessions, ten viz., 001, 002, 003, 004, 005, 006, 011, 015, 018, and 025 were obtained above the mean value. Hence, these ten accessions can be considered as productive accessions.

Six commercially exploited populations of the Indian eri silkworm collected from different parts of north east India analyzed for genetic polymorphism showed

variability among the populations. Hundreds ISSR primer (UBC set # 9) were initially were screened for different populations. Twenty primers, which produced robust and reproducible products, were used for a detailed study on two populations (30 individuals each)SRI-006 and SRI 003. Eighty one per cent of the amplification products from SRI-006 and 70% from SRI-003 were found polymorphic and indicate within population genetic diversity. Total heterozygosity was found as 0.2957±0.0363 and 0.2934±0.0420, respectively and Nei's (1973) gene diversity as 0.2957±0.19404 and 0.2934±0.2050, respectively for SRI-006 and SRI-003 population.

A preliminary study on the DNA fingerprinting by Vijayan *et al.* (2006) using the six eco-races (001 to 006) (Fig 3-7)suggested that the gene flow between the populations of 003 and 005 is quite high (0.9035) and lowest (0.2172) is between 002 and 003, but have similar in phenotypic traits, such as cocoon colour. The genetic distance is lowest between 003 and 005 (0.0654) and highest between 002 and 006 (0.3811). Within populations heterozygosity is higher in 001 (0.1093) and lowest in 002 (0.0510). The high co-efficient of gene differentiation value (0.657) among populations combined with low gene flow contributes significantly to the genetic differentiation among *S. ricini* populations. The high phenotypic and genetic similarity as well as gene flow between populations of 002 and 005 suggests its common origin and later progression into different populations by adapting to the varying climatic conditions.

DNA extraction of individuals of populations of 10 eco-races have been completed following the phenol: chloroform extraction method. DNA samples of population of 5 eco-races were quantified and analyzed using Inter Simple Sequence Repeats (ISSR) primers. Primers were designed from the cDNA of known genes of lepidopteran insects and to be used for amplification of various populations to study the polymorphism at genetic level. Sixty primers produced monomorphic profile in different populations while 53 primers revealed polymorphism within the populations. Primers with (CT)5, (AG)5 and (GA)8 sequences showed high levels of polymorphism.

The present study revealed that as in other Lepidoptera there exist minor variations within single population of *Samia ricini.* According to Hawkes (1918), characters such as large black mottles on the integument, blue vs. white integument and colour of haemolymph (yellow vs. white) are apparently controlled by single alleles. Wu (1962), who made numerous crosses of *S. ricini*, black mottling is dominant over lack of markings, blue integument is dominant over white, and yellow haemolymph is dominant over white. He also found that the gene for white integument combined with the gene for yellow haemolymph resulting in larvae that had yellow instead of white integument.

With the landmark achievement in eri silkworm research and development, to face emerging challenges of WTO and IPR gaining importance the world over, collection, conservation and evaluation of eri silkworm diversity for future breeding is the foremost task. However, DNA fingerprinting will provide a distinct, stable and uniform method of variety identification. With improved varieties, all parental lines and important accessions will be characterized by molecular techniques.

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Table 1. List of passport data of different collection of eri germplasm, *Samia ricini*

Sl. No.	Accn No.	Donor	Origin	Class	Parentage
1	SRI-001	RERS, MEG	ASM	O(RCU)	OR
2	SRI-002	RERS, MEG	ASM	O(RCU)	OR
3	SRI-003	RERS, MEG	ASM	O(RCU)	OR
4	SRI-004	RERS, MEG	MEG	O(RCU)	OR
5	SRI-005	RERS, MEG	MEG	O(RCU)	OR
6	SRI-006	RERS, MEG	MEG	O(RCU)	OR
7	SRI-007	CMERTI, ASM	NAL	N	OR
8	SRI-008	CMERTI, ASM	ASM	N	OR
9	SRI-009	CMERTI, ASM	ASM	N	OR
10	SRI-010	CMERTI, ASM	ASM	N	OR
11	SRI-011	CMERTI, ASM	MEG	N	OR
12	SRI-012	CMERTI, ASM	ASM	N	OR
13	SRI-013	CMERTI, ASM	ASM	N	OR
14	SRI-014	CMERTI, ASM	ASM	N	OR
15	SRI-015	CMERTI, ASM	MAN	N	OR
16	SRI-016	CMERTI, ASM	ASM	N	OR
17	SRI-017	CMERTI, ASM	ASM	N	OR
18	SRI-018	RERS, MEG	MEG	N	OR
19	SRI-019	CMERTI, ASM	ASM	N	OR
20	SRI-020	CMERTI, ASM	NAL	N	OR
21	SRI-021	CMERTI, ASM	ASM	N	OR
22	SRI-022	CMERTI, ASM	MEG	N	OR
23	SRI-023	CMERTI, ASM	ARP	N	OR
24	SRI-024	CMERTI, ASM	ASM	N	OR
25	SRI-025	CMERTI, ASM	ASM	N	OR
26	SRI-026	CMERTI, ASM	ASM	N	OR

SRI – *Samia ricini*, RERS – Regional Eri Research Station, CMERTI – Central Muga Eri Research & Training Institute, MEG – Meghalaya, ASM – Assam, NAL – Nagaland, MAN – Manipur, ARP – Arunachal Pradesh, O – Old, RCU – Race in current use, N – New, OR – Original Race.

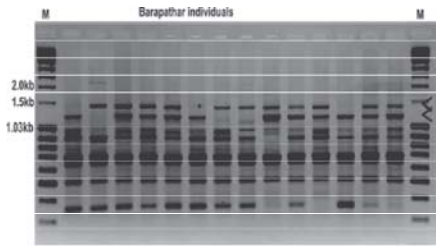

Fig. 3 PCR profile generated by ISSR primer 840 with eri individuals of SRI-009 population

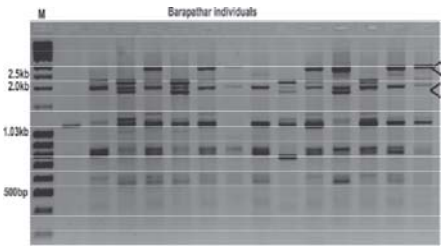

Fig. 4 PCR profile generated by ISSR primer 885 with eri individuals of SRI-009 population

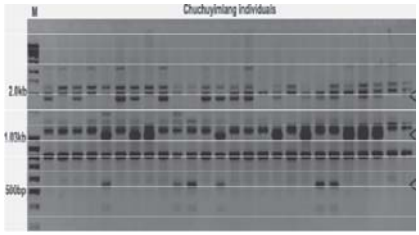

Fig. 5 PCR profile generated by ISSR primer 886 with eri individuals of SRI-001 and SRI-003 population

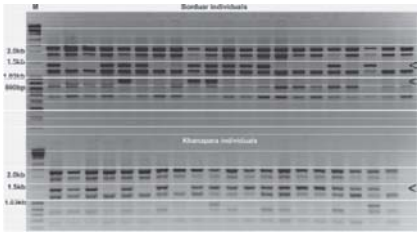

Fig. 5 PCR profile generated by ISSR primer 886 with eri individuals of SRI-007 population

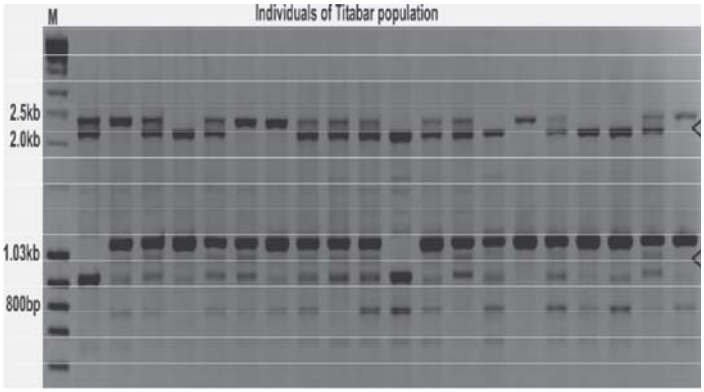

Fig. 7 PCR profile generated by ISSR primer 811 with eri individuals of SRI-002 population

Table-2. Morphological traits of eri germplasm.

Sl. No.	Accn. No.	Larval body colour	Cocoon colour	Fecundity	Hatching (%)	Larval wt. (g)	Larval period (days)	ERR (%)	Cocoon wt. (g)	Shell wt. (g)	Shell Ratio (%)
1	SRI-001	Plain & Zebra on Yellow and blue	White	441.99	95.04	8.27	23	90.06	3.64	0.5	13.74
2	SRI-002	Plain & Zebra on Yellow and blue	White	458.91	94.35	8.25	22	90.52	3.70	0.48	12.97
3	SRI-003	Plain yellow and blue	White	435.46	94.05	7.31	23	88.12	3.55	0.48	13.52
4	SRI-004	Plain yellow and blue	White	442.74	92.56	8.47	21	87.31	3.52	0.46	13.07
5	SRI-005	Plain blue	White	455.44	91.77	8.42	23	89.25	3.61	0.47	13.02
6	SRI-006	Plain yellow and blue	White	459.50	92.61	8.43	22	88.24	3.56	0.47	13.20
7	SRI-007	Plain yellow	White & brick red	385.44	76.89	7.97	22	85.22	3.79	0.42	11.08
8	SRI-008	Plain & Zebra on Yellow and blue	White	413.00	78.90	7.73	23	82.00	3.13	0.39	12.46
9	SRI-009	Plain & Zebra on Yellow and blue	White & brick red	345.00	71.20	7.92	22	80.00	3.32	0.40	12.05
10	SRI-010	Plain & Zebra on Yellow and blue	White	418.50	90.42	8.00	23	87.50	3.10	0.38	12.26
11	SRI-011	Plain yellow and blue	White	366.50	89.33	8.11	21	85.25	3.56	0.45	12.64
12	SRI-012	Plain & spotted on yellow & blue	White	318.50	86.78	8.14	22	90.50	3.10	0.36	11.61
13	SRI-013	Plain & Zebra on Yellow and blue	White & brick red	385.00	84.92	7.62	22	81.50	3.24	0.37	11.42
14	SRI-014	Plain yellow and blue	Brick red	348.00	79.50	7.80	23	76.65	3.87	0.46	11.89
15	SRI-015	Plain yellow and blue	White	414.75	91.40	8.19	21	79.50	3.62	0.44	12.15
16	SRI-016	Plain yellow and blue	Brick red	340.40	89.56	8.17	21	88.65	3.08	0.37	12.01
17	SRI-017	Plain yellow and blue	White & brick red	371.50	87.45	7.79	23	86.50	3.17	0.38	11.99
18	SRI-018	Plain yellow and blue	White	496.41	92.20	8.48	22	89.96	4.51	0.59	13.08
19	SRI-019	Spotted on yellow	White	357.50	85.04	7.64	23	90.06	2.95	0.37	12.54
20	SRI-020	Plain yellow	White	472.00	84.35	8.19	24	90.52	3.09	0.38	12.30
21	SRI-021	Plain yellow	White	353.00	84.05 7.48	25	88.12	3.62	0.49	13.54	
22	SRI-022	Plain yellow	White	442.74	72.56 8.14	25	87.31	3.11	0.40	12.86	
23	SRI-023	Plain yellow	White	255.44	91.77 8.11	25	89.25	3.24	0.41	12.65	
24	SRI-024	Plain yellow and blue	Brick red	359.40	82.61	8.00	25	88.24	2.85	0.35	12.28
25	SRI-025	Plain yellow	White	430.00	94.00	8.43	18	85.00	3.01	0.45	14.95
26	SRI-026	Plain yellow and blue	Brick red	380.00	92.00	6.65	20	92.00	2.00	0.30	12.55

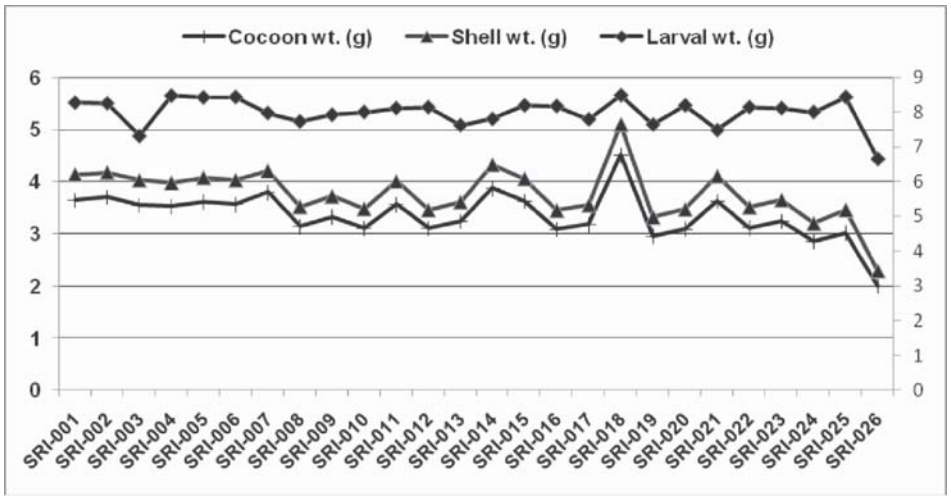

Fig. 1. Post embryonic characteristics (larval wt., cocoon wt., and shell wt. in g) of eri germplasm.

Fig. 2. Hatching (%), Shell ratio (%) and Effective rate of rearing (%) of eri germplasm.

Table: 3. Summary of statistical analysis.

Sl. No.	Statistics	Fecundity	Hatching (%)	Larval wt. (g)	Larval period (day)	Effective Rate of Rearing	Cocoon wt. (g)	Shell wt. (g)	Shell Ratio (%)
1	Mean (S.E)	403.3269 (13.8195)	87.1273 (1.3276)	7.9888 (8.135)	22.4615 (0.3198)	86.8165 (0.7702)	3.3785 (7.362)	0.4238 (1.205)	12.6088 (0.1593)
2	Minimum	255.44	71.20	6.65	18.00	76.65	2.85	0.30	11.08
3	Maximum	496.41	95.04	8.48	25.00	92.00	4.51	0.59	14.95
4	Range	380.35	23.84	1.83	7.00	15.35	1.66	0.29	2.66
5	Standard deviation	70.4660	6.7692	0.4148	1.6305	3.9275	0.3754	6.145	0.8122
6	Variance	4965.451	45.822	0.172	2.658	15.425	0.141	3.777	0.660
7	Skewness (S. E.)	1.082 (0.456)	-0.968 (0.456)	-1.466 (0.456)	-0.466 (0.456)	-1.144 (0.456)	0.999 (0.456)	0.495 (0.456)	0.722 (0.456)
8	Kurtosis (S.E)	3.954 (0.887)	0.105 (0.887)	3.085 (0.887)	1.094 (0.887)	0.615 (0.887)	1.666 (0.887)	0.770 (0.887)	1.578 (0.887)

Bioenergetics and commercial productivity of Eri silkworm *Samia ricini* Donovan in different temperature conditions.

A. Vijaya Bhaskara Rao* S.Smitha*, P.Jaya Prakash, and N. Suryanarayana***.**
***Department of Sericulture Sri Krishnadevaraya University, Anantapur-515003 India.**
****Regional Tassar Research Station, Central Silk Board, Warangal-506009.Director,**
*****Director, Central Tassar Research Institute Ranchi, India.**

ABSTRACT

Of the different problems of ericulture, environmental factors are important for sustainable productivity. Ambient temperature plays a very important role with tremendous influence on the eco physiology of insects including eri silkworm. Eri silkworms are Ectothermic, where body temperature conforms to the external. Bioenergetics involves the various parameters such as food ingestion, faeces defecated, total food assimilated, total food oxidized and total food converted. These parameters were correlated with the commercial productivity at different temperature conditions. In eri silkworm it has been established that high temperature 34^0C and low temperature 20^0C adversely effected the rearing as well as commercial productivity.

Key words: Eri Silkworm, Castor plant, *Ricinus communis*, Temperature, Consumption, Food utilization Budget, Oxidization, Commercial productivity.

INTRODUCTION

Food and growth are active dynamic process with feed back mechanisms and wide ranging ramification through out an insect life. Studies on consumption, digestion and food utilization in insects are of fundamental importance for proper understanding of nutrition in insects (Waldbauer, 1964). Bioenergetics involves in various parameters such as food ingestion, faeces defecated, total food assimilated, total food oxidized and total food converted. Scriber and Slansky (1981) emphasized the importance of the experimental determination of food utilization budgets which are known to offer most important clues in regard to success of terrestrial animals. Several authors have studied the effect of temperature on food utilization budgets (Muthukrishnan, 1985; Reynolds and Wottingham, 1985; Premaleela, 1986;). Acclimation to higher or lower temperature involves changes in food consumption (Hochchka and Somero,1973; Wieser,1973, May,1979). It is well known that higher temperature within bio-kinetic range accelerate the rate of energy transformation but not the efficiency (Kinne,1970; Delvi and Pandian, 1971;1972; Muthukrishnan and Delvi, 1974). The literature available on bioenergetics and commercial productivity in different temperature conditions is scanty. As there is availability of enormous host crop potentialities in semi arid zone where the temperature is ranging from 18^0C to 49^0C in different agro climatic regions of India, it is the need of the hour to study various bioenergetic parameters correlating with productivity and cocoon commercial characters at different ambient temperatures in IV & V instars of Eri silk worm *Samia Ricini* which will pave the way for profitable Eri culture.

MATERIALS AND METHODS

Rearing activities were experimented with 25 disease free slayings each experiment was replicated 5 times @ 05 Dfl per replication. Trays-cum-shelf rearing method suggested by Jaya Prakash et.al.(2006), was followed for assessment of cocoon productivity.Rearing room temperature was maintained at 25^0C±1,20^0C±1 and 34^0C±1. For assessment of commercial productivity performance, data on effective rate of rearing (ERR), cocoon weight, cocoon no. per kg, green cocoon harvest (by nos, and weight) was recorded.

Bioenergetics involves the various nutritional parameters such as food ingestion, faeces defecated, total food assimilated, total food oxidized and total food converted. These nutritional parameters were estimated by the method followed by Waldbauer (1968) and Delvi and Pandian (1972). The food and the faeces defecated by the erisilkworm were weighed in an electronic single pan balance (Metler) to an accuracy of 0.01 mg. Faeces and food were dried in an oven at 90^0C till the weight constancy was attained. Consumption (food intake) was determined by subtracting the dry weight of uneaten food from the dry weight of the food provided (Waldbauer,1968), All faeces were daily separated at 6.00AM from the uneaten food before offering the first feeding and its dry weight was taken as measurement for excretion. Dry food assimilated by the test individuals, during the IV & V instars was calculated by subtracting the dry weight of the faeces produced from the dry food consumed. Assimilation and conversion were calculated by the method followed by Delvi and Pandian (1972). The total amount of food converted into body substance was calculated by subtracting the dry weight of the individual before the experiment from the dry weight of the individual after the experiment. Food oxidized was calculated by subtracting the food converted from the food assimilated. Food utilization budget of eri silkworms was studied using IBP terminology (Petrusewicz and Mac Fadeyan 1970).The statistical analysis of data was carried out as per the methods suggested by Fischer and Yates (1963).

RESULTS AND DISCUSSION

The food consumption/ingestion (food intake) is found to be higher in the V instar compared to IV instar. In both instars, soon after moulting the individuals began to feed without any post-moult fasting period as observed in other insects (Delvi, 1972). As far as the effect of temperature is concerned, when compared to optimum temperature (25^0C) (Table.1) the food intake is slightly increased at higher temperature but decreased at lower temperature in both the instars. The variation defecated (excreted) in IV and V instars correlate with the same trend as in the case of food intake.

At optimum temperature, the food oxidized was found to be more in the V instar than IV instar. As the temperature decreases the oxidization of food increases in both the larvae, exhibiting and inverse relationship between the food oxidized and ambient temperatures (Table.1). The variation in the food-assimilated values followed the same trends as seen in the food oxidized. Food conversion values also followed the same trends as seen in the food assimilated. It is interesting to note that any sudden drop in feeding or increase in feeding resulted in either decrease or increase in feeding and resulted in either

decrease or increase in the assimilation efficiency. The results are in conformity with findings of Naik, (1985). The rise in temperature accelerated the metabolic activity of the silkworm while it is slacking when the temperature goes down. Hence, at higher temperature the larval growth is quickened and consequently the larval period is shortened. On the other hand at lower temperature, the growth is slowed down and the larval period is prolonged. The levels of the physiological and bio-chemical parameters are enhanced at lower temperature. It is well known that the lepidopteran insects accumulate sufficient food energy during the larval period to tide over the non-feeding pupal and adult stages (Delvi and Pandian, 1972). The reduced consumption at lower temperature may be due to enhanced larval period with the decrease in temperature under controlled condition. In other words, the feeding rate increased with the increase in temperature in the present investigation under controlled condition. The increase in temperature most probably not only increases the food intake but also the rate of enzyme secretions and its activity. According to Kinne (1970), higher temperature within bio-kinetic range accelerates the rate of energy transformation but not the efficiency.

It is clearly evident from the temperature studies are in agreement with the results of productive performance and commercial characters of eri-cocoon. The temperature variations has influenced the larvae of *Samia ricini*, eri silkworm by increased/decreased commercial characters (Table.2) like (cocoon weight, pupal weight, shell weight, silk ratio) and productive performance parameters such as (Table.2) Good cocoons harvested (%), Effective rate of rearing (%), Average weight of cocoons (gms), No.of cocoons per Kg, No. of green cocoons, Wt.of green cocoon (Kgs), Wt. Of dry shells (Kgs). On the whole it is observed that the cocoon productivity and cocoon commercial characters were decreased with low temperature of 20^0C and high temperature at 34^0C. On the other hand the optimum temperature of 25^0C is more favorable for qualitative and quantitative eri cocoon production by utilizing efficient bioenergetics mechanisms in eri silkworm.

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Table. 1. Comparison between IV & V Instars in the levels of food budget i.e. consumption, excretion, assimilation, conversion and oxdization of eri silkworm, *Samia Ricini* fed on caster leaves, *Ricinus Communis* (ad libitum) at different ambient temperature conditions

± Standard deviation.

Instar Parameter	IV Instar			V Instar		
	Opt. Temp.	Lower Opt.	Higher Temp.	Opt. Temp	Lower. Temp	Higher Temp.
	25^0C	20^0C	34^0C	25^0C	20^0C	34^0C
Consumption	685.00 ± 41.82	575.00 ± 39.63 (-16.5)	820.00 ± 44.00 (+19.7)	3860.00 ± 395.40	3240.90 ± 360.02 (-19.13)	4140.00 ± 410.30 (+7.25)
Excretion	240.00 ± 26.92	220.75 ± 24.98 (-8.02)	305.00 ± 28.22 (+27.0)	1925.00 ± 151.23	1990.30 ± 150.00 (+3.39)	2210.00 ± 154.29 (+14.80)
Assimilation	455.00 ± 28.34	354.25 ± 26.23 (-22.19)	515.00 ± 29.67 (+13.18)	1935.00 ± 146.36	1250.60 ± 138.84 (-35.36)	1900.00 ± 150.92 (-1.50)
Conversion	86.90 ± 8.03	68.70 ± 6.82 (-19.9)	81.20 ± 7.84 (-6.55)	160.00 ± 18.20	146.90 ± 16.90 (-8.75)	178.25 ±19.50 (+11.40)
Oxidization	368.10 ± 30.99	285.55 ± 28.84 (-22.42)	433.80 ± 32.32 (+17.84)	1775.00 ± 125.40	1103.70 ± 114.38 (-37.85)	1721.75 ± 113.60 -3.0

Values are expressed in mg/wet wt/instar/larva
Each value is a mean of eight estimations
Percentage increase (+) / decrease (-) relative to optimum temperature is given in parenthesis

Table. 2. Productive performance and Commercial characters of Eri cocoons,fed on caster leaves,Ricinus communis(ad libitum) at different ambient temperature. ± Standard deviation

Parameters	Different Temperatures		
	25^0C	20^0C	34^0C
1. Good Cocoons Harvested	93.00 ± 3.40	82.13 ± 5.71 (-11.68)	57.32 ± 3.30 (-38.36)
2. ERR	74.56 ± 1.32	67.02 ± 4.00 (-10.11)	39.50 ± 2.32 (-47.02)
3. Average Weight	2.95 ± 0.09	3.05 ± 0.21 (-3.38)	2.26 ± 0.20 (-23.38)
4. No. of Cocoons/Kg	339 ± 13.00	328 ± 15.00 (-0.29)	442 ± 21.00 (+ 30.38)
5. No. of Green Cocoons	21642 ± 1171	15580 ± 975 (-28.01)	8221 ± 1543 (-62.01)
6. Wt. Of Green Cocoons	63.840 ± 0.52	47.500 ± 0.112 (-25.59)	18.600 ± 0.17 (-70.86)
7. Wt. Of Dry Shells	14.00 ± 0.13	8.830 ± 0.85 (-36.92)	3.150 ± 0.23 (-77.5)
8. Cocoon Weight (gm)	3.00 ± 0 .09	3.05 ± 0.09 (+ 1.66)	2.26 ± 0.21 (-24.66)
9. Pupal Weight (gm)	2.56 ± 0.10	2.62 ± 0.15 (+ 2.34)	1.970 ± 0.12 (-29.94)
10.Shell Weight (gm)	0.44 ± 0.09	0.43 ± 0.10 (- 2.27)	0.290 ± 0.15 (-34.09)
11. Silk Ratio (%)	14.67 ± 0.46	13.90 ± 1.25 (- 5.24)	12.83 ± 0.55 (- 12.54)

Each value is a mean of eight estimations
Percentage increase (+)/ decrease (-) relative to optimum temperature is given in parenthesis.

Biodiversity of Muga Silkworm Antherea Assamensis Helfer (Lepidoptera, Saturniidae) and their Morpho-Molecular Variations.

R. Chacravorty[1], K. Neog[1], A.K. Sahu[2], R. Singh[3] and B.G. Unni[3]. 1: Central Muga Eri Research & Training Institute, Lahdoigarh, Jorhat, PIN: 785700, Assam (India). 2. Regional Muga Research Station, Boko, Kamrup, Assam (India). 3. North East Institute of Science & Technology, Jorhat, Assam, (India).

ABSTRACT

Two cultivated and four wild muga silkworm (*Antheraea assamensis*) populations were collected and maintained by the Central Muga Eri Research & Training Institute, Jorhat, Assam, India. The goals were to characterize the strains morphologically and also at molecular level using SSR, microsatellite and RAPD markers. All the populations possessed distinct morphological characteristics although little variations were observed in qualitative characters. The entire marker assisted DNA analysis of the populations indicated within and between population diversity nullifying the popular notion of mono-racial status of the silkworm species. This has also clearly indicated possibility of genetic improvement of the insect through molecular breeding approaches.

Key words: *Antheraea assamensis*, morphological characters, DNA markers, genetic diversity

INTRODUCTION

Muga silkworm, the producer of natural shimmering golden yellow colour silk, is the pride of northeast India in general and Assam in particular. Assam very recently has obtained the possession of this precious silkworm as the geographical indication (GI) under IPR. On economic front, at present, about 7570 hectares of land is under Muga food plantation, and annual production of raw silk in the region is 100 MT. More than 30,000 families are engaged in Muga culture directly and more than 1 lakh families are involved in the post cocoon sector making it a profit making industry in the region.

Since, culturing of muga in its larval stage is done out-door, it is exposed to different environmental conditions and attack of parasites, predators and diseases. It results in heavy losses due to weather conditions, pests, diseases and natural calamities. Nevertheless, barring all the natural, muga rearers have been practicing this culture by collecting eggs from other source or prepare eggs from the moths emerging out from a selected portion of cocoons from previous crop. Thus, there was always a selection pressure on the muga populations for obtaining healthy seed source. Since, there were no such morphological characters distinguishing one brood of worms to other, rearers selected seed cocoons with utmost care based on size of cocoons, habit of the larvae, response to physical touch, eating behaviour *etc*. Based on their long lasting experiences, the rearers of Assam classified the muga worms in local parlance as borbhogia, sorubhogia *etc*. (Choudhury, 2005). But till now, a definite race or variety of the silkworm is not available with distinct morphological, anatomical or rearing behaviour. This has led to prevalence in

the mind of common people as well among scientific community to think muga silkworm to have a mono-racial status without any genetic divergence among or between the populations rearing throughout the length and breadth of the northeastern region of the country and also available in wild habitat. But, this status questions the very existence of this species through time immemorial, vagaries of weather and seasons, diseases, pests *etc*.

There is report of presence of some colour variations among the silkworm populations (Chakravorty *et al.,* 2006), these variations are not static in the sense that the same colour may not appear in the next generation (s). No records have so far made indicating diversity of the species at morphological level or at molecular level till now. A number of references can be cited regarding assessment of genetic diversity of different organisms including sericigenous insects like *Bombyx mori*, *Antheraea mylitta* and *Samia ricini* (Nagaraja & Nagaraju, 1995; Promboon *et al*., 1995; Singh, 1997; Thanananta *et al*., 1997; Nagaraju, 2002; Murthy *et al*., 2006; Vijayan *et. al*., 2006). But such works on the culturally and economically important muga silkworm are yet to be recorded. In this paper attempts are made to give a brief outline of the study undertaken at CMER&TI, Lahdoigarh to verify the existence of genetic variability between and among selected muga silkworm populations.

MATERIALS AND METHODS

Two cultivated and four wild stocks of muga silkworms (designated as Population-1 to Population -6) were collected from different prominent muga growing areas of Assam and Meghalaya, *viz*., adjoining areas of Jorhat, Assam and Tura, Meghalaya, India. Only cocoons were collected from each site and dfls were prepared in the Institute following standard procedures. Five dfls of each accession were kept for hatching and newly hatched worms were brushed on leaves of Muga silkworm host plants maintained in the experimental field of the institute. Since, Muga host plant “Som” is the most preferred one (Chakravorty *et. al*., 2004a), brushing of the worms were done on these plants only. Maintenance of the host plants and rearing of silkworms were done following standard procedures (Chakravorty *et al*., 2005). Morphological characters of the silkworms were recorded during the growth period at different stages as per descriptor (Chakravorty, *et al*., 2004b).

The DNA was extracted from 5th instar silkworm larvae following the method of Thanananta *et al*. 1997; Nagaraja & Nagaraju 1995. The genomic DNA was quantified on 0.8% Agarose gels and diluted to uniform concentration (10ng/µl) for marker analysis. Three populations of muga silkworm (Population – 1, Population-2 and Population-3 from upper Assam, lower Assam and Garo hills) were analyzed using 15 SSR primers. 37 microsatellite primers provided by CDFD, Hyderabad were successfully tested by Polymerase Chain Reaction (PCR) with the extracted *Antheraea assama* DNA from all the six populations. PCR based molecular marker, random amplified polymorphic DNA (RAPD) technique was also used to study the DNA profiles of the muga silkworm populations.

RESULTS AND DISCUSSION

A. Morphological characterization:

Morphological characters of the collected populations of muga silkworm in respect of egg, larva, cocoon, pupa and moth are presented in Table 1. The ratios of length and breadth of eggs varied from 2.0:1.8 in Population-1 to 2.30x 2.1in Population-5, weight of eggs varied from 6.8 to 7.50 mg. Eggs were streakless and incubation period varied from 7-10 days. Wide range of variations was recorded in length, breadth and weight of larvae. Length, breadth and weight of male larvae were comparatively lower than that of females in all the populations and there were variations among the populations in respect of these characters. No significant variation in cocoon colour, shape, texture, nature of floss *etc*. were observed except in the cases of peduncle length, cocoon breadth, cocoon and shell weight of the populations. Maximum peduncle length was recorded in the female cocoons of Population-5 (5.50cm). Length, breadth and weight of pupae were much higher in females compared to males. Highest female and male pupal weight was recorded in Population-6 (4.80 g and 8.20 g, respectively). In case of moth characters, highest range of male and female body length was recorded in Population -6 (4.82 cm and 5.60cm, respectively); maximum wing span in Population -6 (15.0 cm in males and 17.00 in females).

B. Molecular characterization

Three populations of muga silkworm from upper Assam, lower Assam and Garo Hills were analyzed using 15 SSR primers. Out of which nine primers showed amplification at the expected locus size. The locus showed homozygocity within and between Population-1 and Population-2. Population-3 showed heterozygocity exhibiting polymorphic loci on PAGE gel resolution. The SSR locus of AaSat 01, 02, 08 and AaSat 20 showed allelic polymorphism in Population-3 where AaSat 10 was absent. Amplification with 13 ISSR also generated within population polymorphism. The percentage of polymorphic loci in the Population-5 was 91.67% (Table 2). Details of microsatellite analysis of these three muga populations are shown in Fig.1.

Further, thirty seven microsatellite loci developed at CDFD, Hyderabad were screened for polymorphism in six different genotypes of muga silkworm. Of these, eleven SSR loci showed **"within genotype"** and "**in between genotype"** polymorphism. Population - 3 showed presence of multiple alleles in the loci **AaSat** – 1, 2, 8, 20, 53, 44, 40 and 14 indicating high level of "**within"** polymorphism. Aasat-14 showed monomorphic pattern across the populations except in population-3. Population-4 and Population-6 were polymorphic in these loci which indicated low **"within population"** genetic diversity. AaSat-59 showed multiallelism in Population – 3 **(Table-3).**

Since RAPDs are random selection of DNA sequence, it was apparent in the study so far in Figure 2, that the RAPD technique was sensitive enough to detect differences between populations of muga silkworm in which differentiation is not always possible morphologically. The strains were quite distinct in their RAPD profiles. All the populations generated 3-4 bands which were distinct and polymorphic. It has been observed that the wild muga strains were more high-yielding than the domesticated strains. So these wild strains can be explored to enhance the silk fibre production in silk industry. Moreover, presence of colour variations at larval stages and subsequent disappearance or

reappearance in the populations may be attributed to presence of jumping genes or transposable elements.

Muga silkworm being found and cultivable profitably only in northeastern states of India has distinct desirable characteristics over other silk producing insects due to its most durable and lustrous silk. But with the shrinkage of its natural habitat, depletion of its population at wild state is reported (Thangavelu and Sinha, 2006; Sahu, 2006). Moreover, from time immemorial muga rearers have been practicing collection of muga silkworm populations from its wild habitat to have robust and successful crops. Collection and characterization of these populations morphologically and also at molecular level is an important step for assessing biodiversity which in turn is a prerequisite for developing a sustainable conservation programme. The present study reflects the efforts made in this line by the working group in the institute with the ultimate objectives of conservation and genetic improvement of these elite but yet endangered species of silkworms.

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Table 1. Morphological characters of the collected populations:

Particulars	Pop.-1	Pop.-2	Pop.-3	Pop.-4	Pop.-5	Pop.-6
A. Egg characters						
Size (LxB mm)	2.00 x 1.8	1.90 x 1.75	2.10 x 1.90	2.10 x 2.0	2.30 x 2.10	2.20 x 2.10
Weight (mg)	7.00	6.80	7.50	7.40	7.50	7.50
Streak present / absent	Absent	Absent	Absent	Absent	Absent	Absent
Incubation period (days)	8-10	7-10	8-10	8-10	8-10	8-10
B. Larval Characters						
5th instar body colour	Green	Green	Green	Green	Green	Green
Length of 5th instar male larva (cm)	8.0	8.2	8.2	8.5	8.50	8.6
Length of 5th instar female larva (cm)	11.2	11.40	10.5	12.5	12.0	13.5
Breadth of 5th instar male larva (cm)	1.20	1.30	1.20	1.50	1.60	1.80
Breadth of 5th instar female larva (cm)	1.60	1.60	1.70	1.80	2.0	2.00
Tubercle number	69	69	69	69	69	69
Number of setae in tubercle	460	460	460	460	460	460
Weight of mature male worms (g)	11.50	10.50	9.00	12.00	11.35	14.00
Weight of mature female worms (g)	14.10	12.20	10.50	15.20	15.15	16.50
C. Cocoon characters						
Peduncle length (male) (cm)	2.50	3.75	2.80	3.80	5.00	4.20
Peduncle length (female) (cm)	3.60	3.80	4.20	4.00	5.50	5.40
Cocoon length (male) (cm)	4.20	4.20	4.80	4.50	4.75	4.80
Cocoon Breadth (male) (cm)	2.00	2.00	1.90	2.10	2.00	2.10
Cocoon length (female) (cm)	5.10	4.80	5.00	5.40	5.32	5.40
Cocoon Breadth (female) (cm)	2.20	2.00	2.00	2.50	2.30	2.50
Cocoon weight (male) (g)	4.30	4.00	3.80	4.50	4.95	4.50
Cocoon weight (female) (g)	8.00	7.80	7.85	8.40	8.30	8.40
Shell weight (male) (g)	0.36	0.35	0.35	0.38	0.45	0.48
Shell weight (female) (g)	0.48	0.50	0.45	0.60	0.70	0.74
D. Pupal characters						
Length of male pupa (cm)	3.8	3.80	4.00	4.00	4.10	4.20
Length of female pupa (cm)	4.50	4.60	4.8	5.00	5.00	5.20
Breadth of male pupa (cm)	1.40	1.50	1.50	1.70	1.70	2.00
Breadth of female pupa (cm)	1.80	1.85	2.00	2.20	2.20	2.40
Weight of male pupa (g)	4.00	4.25	4.50	4.20	4.50	4.80
Weight of female pupa (g)	6.80	7.00	7.20	7.80	7.60	8.20
E. Moth characters						
Body length of male moth (cm)	4.10	4.20	4.00	4.30	4.60	4.82
Body length of female moth (cm)	5.30	5.00	4.80	5.50	5.50	5.60
Male wing span (cm)	12.30	12.00	12.50	14.00	14.50	15.00
Female wing span (cm)	13.50	13.00	14.60	16.20	16.50	17.00
Length of antennae of male moth (cm)	1.45	1.20	1.64	1.40	1.72	1.70
Length of antennae of female moth (cm)	1.42	1.25	1.30	1.80	1.50	1.60
g. Breadth of antennae of male moth (cm)	0.60	0.55	0.60	0.80	0.70	0.80
Breadth of antennae of female moth (cm)	0.42	0.40	0.40	0.50	0.50	0.50

Table 2. Genetic diversity parameters showing within population genetic variability in different populations of *A assamensis*.

Locus	Sample Size	na*	ne*	h*	I*
Population -3					
Mean	16	1.8611	1.6053	0.3461	0.5065
SD		0.3507	0.3228	0.1616	0.2243
Polymorphic Loci : 86.11%					
Population-2					
Mean	16	1.9167	1.5531	0.3253	0.4867
SD		0.2803	0.3225	0.1554	0.2664
Polymorphic Loci : 91.67%					
*na = Observed number of alleles *ne = Effective number of alleles *h = Nei's (1973) gene diversity. * I = Shannon's Information Index					

Table 3. Occurrence of alleles in four wild populations of muga silk worm.

Locus	Alleles (Size in bp) present in the population			
	Population-3	Population-4	Population-5	Population-6
Aasat1	188,192, 194, 196, 198, 201, 203	192	192	199
Aasat2	131, 135, 136, 138, 140, 145, 149	148	148	148
Aasat 8	199, 202, 213, 215	215	215	215
Aasat20	174, 185, 187, 192, 195, 197, 202, 203, 213	174	176	178
Aasat 59	199	159, 184, 185, 189, 195, 192, 198, 205, 213	195	195
Aasat 53	204, 207, 210, 213, 215, 218	207	207	207
Aasat 44	192, 194, 197, 203, 206	218	218	218
Aasat 65	151	103, 107, 110,116	118	97, 107, 110, 113, 120
Aasat 40	190, 192, 199, 202, 221	133	133	133
Aasat19	151	155	172	172
Aasat 14	460,464	464	464	464
Aasat 06	223	221	221	221

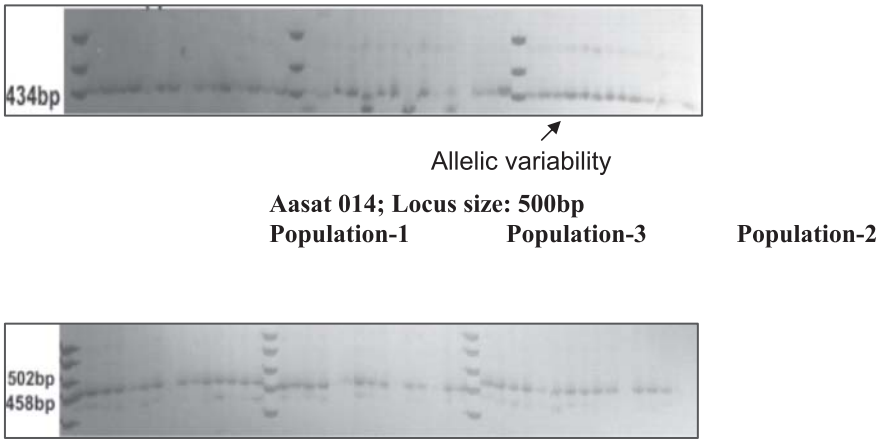

Fig. 1: Microsatellite analysis of three muga populations

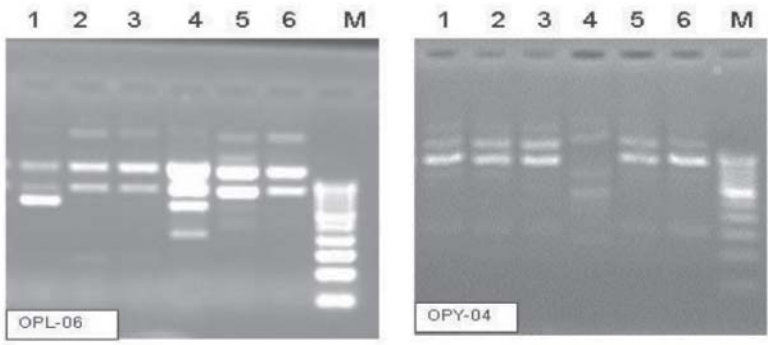

Fig. 2: RAPD profiles of muga silkworm *A. assama* genotypes obtained with primers OPL-06, OPY-04, Serial number of the genotypes are as given in the Table 1. M = Standard DNA marker, 100 bp DNA ladder.

Performance of Eri Silkworm Philosamia Ricini Hutt. Under Agro – Ecological regions of Maharashtra.

Jadhav, .A.D.,* Kalantri ,L. B.,* Hajare, T. N., Patil,N.G.,** Kulkarni, M.K.,*** Undale, J.P.,* Dhamane,S.D.,* Patil, P.G.,**** Balsaraf,A.U.*****and Sathe ,T.V. ***. *Directorate of Sericulture, Govt. of Maharashtra, Umred road, Nagpur-440009,India. **NBSS&LUP,Amaravati Road,Nagpur-440010,India. *** Dept. Zoology, Shivaji University,Kolhapur-416004,India. ***Union Public Service Commission,New Delhi-69, India. ****Ginning Training Centre,ICAR,Amravati Road,Nagpur-440010 ,India. *****Shivaji Education Sanstha, Amravati-India.**

ABSTRACT

Eri Silkworm *Philosamia ricini* Hutt. is the only wild silkworm which is reared indoors on leaves of castor (*Ricinus cumminis* L) and also on Kessaru leaves. Ericulture is mostly confined to north eastern states, Bihar and some parts of Orisa, West Bengal and recently introduced in Andhra Pradesh. Five to Six crops are harvested in a year and normally the larval period varies from twenty days in summer to thirty days in winter. Ericulture recently introduced in Maharashtra with unique advantage of easy rearing , resistant to pest and diseases, castor is extensively cultivated in Maharashtra particularly for production of castor seed. The recent trials on eri silkworm rearing shows that ericulture can give assured income Rs. 7200/- to 12040/- in a short span of time besides income from castor seed which makes ericulture attractive income entrepreneurship for the castor growers of the state. Hence ,attempt has been made to find out feasibility and viability of Eri silkworm *P. ricini* rearing under agro ecological region of Vidarbha and Marathwada region of Maharashtra .Experimental trials were conducted by Directorate of Sericulture of Maharashtra , Nagpur during the years 2006 - 2007. Results of present study indicated the feasibility of ericulture in the state. Cocoon yield per 100 dfls 48.20 kg- 52.373kg and 55.97 kg to 65.47 kg obtained during winter crop and early summer seasons under Vidrabha and Marathwada conditions respectively. The other commercial parameters like ERR%, single cocoon wt., shell ratio % recorded on higher side also indicates the feasibility of ericulture in the State of Maharashtra.

Key words; Eri silkworm, ERR, Castor.

INTRODUCTION

The non mulberry wild silk culture is presently popularized as “Vanya Silk Culture” (Eri, muga and Tasar). The Vanya silks have a higher market value. Recent researches on Vanya Silk indicated that they possess some physiological significance like controlling cholesterol in haemolymph , anti bacterial function and U V absorption effects .India produces all the commercially exploitable silk Mulberry, Eri ,Tasar, Muga and Oak Tasar. Mulberry and Eri are domesticated silkworms. Mulberry sericulture has the highest production and widest distribution in the country, followed by Eri, Tasar, Muga and Oak Tasar culture.

The major share in Non Mulberry group goes to Eri silk . Its production has grown from 168MT in 1971-72 to 1448 MT in 2004-05.This growth rate is higher than that of mulberry.(Suryanarayana *et.al*.,2003) Ericulture can be promoted

as small house hold activity with a small piece of land .Hence , Ericulture has been found to be suitable owing to available castor plantation , climate, technology and test results in the state of Maharashtra, Jadhav, *et.al*. 2004.

MATERIAL AND METHODS

Field trials were conducted during different months at farmer's fields in Nagpur, Yawatmal, districts of Vidarbha and Hingoli / Parbhani districts of Marathwada region. Disease free layings were obtained from Directorate of Sericulture, Assam and Regional Tasar Research Station Warangal, Andhra Pradesh. Proper disinfection of rearing house and appliances was carried out with 5 % bleaching powder solution. Incubation of layings was carried out at farmers house at 26 C & 80 % RH in a suitable room . Hatched larvae of first two days were taken for further rearing . Early stage worms were fed with tender and succulent castor (*R. communis*) leaves variety GCH-4 green by spreading chopped leaves over newly hatched worms. Up to third stage i.e. during chawki rearing a temperature of 26-28 0 C ,80 –90 % Rh was maintained . In later stages worms were fed with whole leaf i.e. whole leaf feeding method. About 30-40 % of the leaves of castor plants were used for rearing . In all the stages proper spacing maintained to avoid over crowding as per the prevailing practice of eri silkworm rearing. To avoid further spread of diseases, weak, injured, diseased and irregular worms were collected periodically and discarded /buried . Matured worms were collected and mounted in the plastic mountage insuring 30-40 worms per sq. feet . During spinning proper aeration was maintained in the mounting room. The cocoons were harvested on 5-6th. day. Important quality /economic parameters with respect to larval period , ERR % (effective rate of rearing), number of cocoons per Kg. , single cocoon weight , shell weight and silk ratio % were recorded for further analysis. Total yield was also recorded and converted to Kg. to get yield per 100 dfls.

RESULTS AND DISCUSSION

Table 1. Rearing performance of Eri silkworm(*P. ricini*) under Vidarbha conditions of Maharashtra.

Sr. No	Seasons	No of Dfls	Avg. fecundity (Nos)	Hatching %	Larval period days	ERR %	Actual yield per/100dfls kg	No of cocoons per dfls (Nos)		Cocoon per Kg (Nos)
1.	Winter	200	320	81	24	81.70	48.28	21176	212	439
2.	Early summer	200	340	83	22	82.30	55.97	23225	232	415

Table 2. Rearing performance of Eri silkworm (*P. ricini*) under Marathwada conditions of Maharashtra.

Sr. No	Seasons	No of Dfls	Avg. fecu-ndity (Nos)	Hatc-hing%	Larval period days	ERR %	Actual yield per/100dfls Kg	No of cocoons per dfls (Nos)		Cocoon per Kg (Nos)
1.	Winter	200	315	82	23	82.7	52.33	21361	214	418
2.	Early Summer	200	342	81	22	84.1	65.47	24160	281	369

The data on hatching percentage ranged from 81 to 83 % in Vidarbha and from 82 to 83 % in Marathwada region. The average fecundity ranged from 315 to 340 per dfls during winter crop(Nov/Dec.) and 340 to 342 egg/dfls during early summer (Feb/March)crop.

It is observed that the average weight of matured larvae varies from 7 – 8 gms and 8 to 9 gms respectively in Vidarbha and Marathwada regions during winter months.

Where as during early summer months wt. of the matured larvae ranges from 7.5 gms to 8.0 gms and 8.0 to-9.1 gms under Vidarbha and Marathwada conditions respectively. The average cocoon wt. and ERR % (survivability) data revealed that average yield varies from 212 cocoons per dfls to 232 cocoons per dfls (Table 1) during winter and early summer months respectively under Vidarbha conditions . Where as under Marathwada conditions yield by number per dfls recorded was 232 and 281 cocoons during winter and early summer months respectively. (Table 2) The little variation in cocoons yield and other parameters is mainly due to the variation in climatic condition of rearing site and quality of fed given to the silk worms. The high ERR percent 81.70 during winter and 82.3 during early summer crop under Vidarbha condition and 82.7 and 84.1 during winter and early summer months respectively under Marathwada conditions is very significant performance for preliminary evaluation in non traditional state like Maharashtra. Bramha *et., al* (2007) have also reported ERR% between 80-84% under Doon valley conditions in eri silkworm rearing..

The average cocoon wt. recorded was 2.28 gms and 2.41 gms during winter and early summer months respectively under Vidarbha whereas 2.45 gms and 2.71 gms during winter and early summer months respectively under Marathwada conditions . The data on average shell wt under Vidarbha conditions shows 0.424 gms and 0.529 gms in winter and early summer months respectively. Under Marathwada conditions the average shell wt. was ranged from 0.519 gms to 0.532 gms. The data with respect to shell % varied from 15.38 to 16.16 in winter and early summer months respectively in Vidarbha crops. However, under Marathwada conditions it ranged from 15.78% to 16.32 % respectively. The results with respect to number of cocoons harvested per Kg. varied from 439 to 415 and 408 to 369 cocoons in winter and early summer months in Vidarbha and Marathwada regions respectively .(Kushwa,*et.al,*2005)have also earlier recorded similar results with respect to

commercial characteristics of eri silkworm rearing under Maharashtra conditions.

The rearing performance data of this study clearly showed that eri silk production is feasible thus, it will provide job opportunities , entrepreneurship development to young enterprisers ,to the rural masses in the State of Maharashtra .Sathe and Jadhav , 2000 , Jadhav *et.al* , 2006,2007,have reported that ericulture is feasible under Maharashtra conditions. The study under agro – climatic region of Maharashtra state exhibited variations in raring period, yield and commercial characters such as single cocoon wt, shell wt, shell ratio and larval period.

Thus, it can be concluded that, ericuluture is suitable in the agro climatic conditions of these two regions of the Sate. The results shown in table no.1 and 2 on commercial parameters of eri silkworm rearing under Vidarbha and Marathwada conditions revealed that eri crop performance of Marathwada region is slightly better as compared to results of Vidarbha region. The possible reason for the difference could be the castor cultivation practices followed in these regions. In Vidarbha castor is cultivated as a monocrop with closer spacing without irrigation fertigation . In Marathwada castor is planted along the bunds of turmeric crop with wider spacing grown as high bush plants. Farmers from this region usually irrigate turmeric crop with application of suitable fertilizer doses .Possibly it ensures better leaf quality over Vidarbha method of caster cultivation. Marginal farmers with limited or no access to resources could be the target group for extending the ericulture technology. Recent decline in acreage of cotton which was otherwise a predominant crop in these regions indicates that the farmers are looking for alternatives. Ericulture can augment the income of farmers and help to maintain the cotton production status of the region. Definite and concerted planning with tech.& proficient efforts in collaboration & coordination with all other relevant departments Viz .Central Silk Board, Directorate of sericulture ,Jayant Oil and derivatives Ltd. Mumbai, Dept. of agriculture and Rural development ,extension wings of agriculture Universities nee d to be exploited which may serve for employment of women ,elderly members of family as well as children.

The present study also the revealed fact that by way of ericulture farmers from Maharashtra can get an additional income of Rs. 7200 /- to 12040/- per acre per year of caster plantation besides income out of sell of castor seed.

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In Vitro Shoot Proliferation of Muga Food Plant Som (Persea Bombycina Kost.).

S. A. S. Rahman, M. C. Sarmah, K. Neog & R. Chakravorty. Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat-785 700, Assam (India).

ABSTRACT

Multiple shoots were induced from nodal segments of Som, *Persea bombycina* Kost. on MS Medium supplemented with BA, KIN, IBA and NAA. The nodal segments from the in vitro developed plantlets could he induced again to produce a large number of harvestable shoots. MS medium supplemented with BA (1.5 mg/l) and 0.4 mg/l KIN was most effective in induction of multiple shoots.

Key words: Nodal segment, explant, axillary bud multiplication, *Persea bombycina*

***Abbreviations*:** BA — 6-benzyl aminopurine; IBA — indole 3-butyric acid; KIN — Kinetin; MS — Murashige and Skoog's medium (1962); NAA — a-naphthalene acetic acid;

INTRODUCTION

North Eastern Region of India is the natural abode for muga silkworm. Though muga silkworm is polyphagous in nature, Som (*Persea bombycina*, Kost) is considered as one of the primary food plant of muga silkworm. Som is a open pollinated perennial tree. There are eight different genotypes of som *viz*., S1, S2, S3, S4, S5, S6, S7 and S8 out of which, S3 and S6 is reported to be the most promising genotypes for muga silkworm rearing. Som is generally propagated through seeds. Due to heterozygous in nature it is difficult to get the true to the type progeny of desired genotypes through seeds. Moreover, vegetative propagation in som is not very much successful. Hence, micrpropagation of som will be of immense importance to address such problem and required quantity of seedling can be made available through out the year.

Preliminary work on the tissue culture of related species like *Persea americana* has been reported by P. A. Cooper (1987) and Barcelo-Munoz *et al* (1999) respectively. Micropropagation from seedling of *Persea indica* Calif. was reported by Nel *et al*. (1982) Successful callus culture of *Persea americana* were reported by several workers (Young, 1983; Scroeder, 1980; Gazit and Blumenfeld, 1971). This paper reports the protocol for *in vitro* shoot proliferation of *Persea bombycina* Kost which can he utilized for the large-scale multiplication of the species.

MATERIALS AND METHODS

Nodal segments were used as explants. The material was collected from 10-12 old plants of experimental garden in a beaker containing ascorbic acid solution (1000 ppm). The explants was washed in running tap water for 30 minutes to remove the dust particles followed by a detergent, Tween-20 (5% v/v) for 5

minutes. Then the explants were treated with ascorbic acid solution (1000 ppm) for 2 hours in a orbital shaker. After 2 hours the explants were washed several times in sterile double distilled water before taking them to the sterile airflow chamber. Surface sterilization was carried out by treating with 0.1 % mercuric chloride solution for 4 min, and then washing with sterile water (4—5 times). After blot-drying on sterilized blotting paper, the explants were finally rinsed with 70% alcohol for 1 min. Nodal segments with one viable bud (1- 2 cm size) were then inoculated into the medium. Contamination free cultures were transferred to fresh media in every 15 days initially and thereafter every 20 days.

Culture medium and condition

MS basal medium (Murashige and Skoog,1962) was used for culture initiation. MS basal medium, supplemented with 0.5mg/l KIN and 2.0 mg/l BA and fortified with 3% (w/v) sucrose, was used for the purpose. The responding cultures were used for the multiplication trials. For multiple shoot induction, MS basal medium supplemented with different concentrations and combinations of cytokinins (BA & KIN) and auxins (IBA & NAA) were used. Medium was fortified with 3% (w/v) sucrose and gelled with 0.8% agar. The pH of the medium was adjusted to 5.8 and the media was sterilized by autoclaving at 1.5 lbs pressure and 121 °C for 20 mm. The cultures were incubated at 25±2 °C under 14/8 h photoperiod under white fluorescent tubes (30—35 mol rn^{-2} s^{-1}).

Observations were taken from 10 replicates per treatment on percentage of culture response and number of multiple shoots per culture after 40 days.

RESULTS AND DISCUSSION

Culture Establishment

Establishment of contamination free nodal explants and the culture response was dependant on the selection of suitable explants and weather condition. Partially mature shoots free from fungus/insect infection/infestation, collected during shinny hours of the day were suitable for the induction of best results. Contamination free shoots were transferred to fresh media in every 15 days initially and thereafter every 20 days. It is very important that subculture interval should not exceed more than 20 days at the initial stages specifically, since it adversely affected further response of the tissue. Bud break was observed within 20—30 days. The responding cultures were transferred to multiplication medium with a different combination of growth regulators. The requirement of cytokinin for the initiation of primary cultures was reported earlier (Huang et al.. 1998).

Shoot multiplication

MS basal medium hardly induced multiple shoots. All concentrations of BA/KIN either alone or in combination facilitate axillary bud initiation (Table:1). This is the first report on *in vitro* shoot proliferation in *Persea bombycina* Kost. BA was the most efficient cytokinin for axillary bud initiation and subsequent proliferation of axillary buds. BA alone at 1.5mg/l concentration induced a mean of 5.5 shoots per nodal explant. Kinetin supplemented medium is at per with BA in induction of multiple shoots, but it leads to callus formation. BA 1.5 mg/l in

combination with 0.4mg/l Kinetin was most effective in axillary bud multiplication which developed a mean of six shoots per node explant. When the concentration of cytokinin increased the number of shootlet initiation decreased is in consonance with the finding of Elangomathavan (2003) in *Orthosiphon spiralis.*

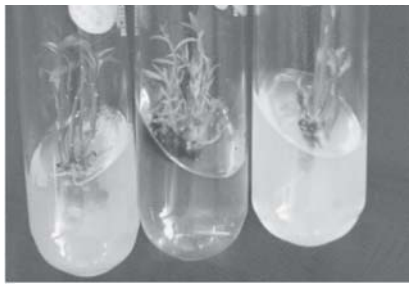

Fig-1. *In vitro* axillary bud multipli-cation of *Persea bombycina* Kost.

BA in combination with auxin also leads to proliferation of axillary buds. BA at 1.5mg/l with 0.2mg/l of IBA and NAA produced a mean of 5.8 and 5.5 shoots per node explant respectively. Similar results were also reported in *Ceropegia candelabrum* (Beena *et al*., 2003),

Table1. Axillary bud multiplication of Persea bombycina on MS medium supplemented with different growth regulators (mg/l).

Sl No.	BA	Kinetin	IBA	NAA	Percent response	*No. of shoot per explant
1	Without growth regulators				50	1.3
2	0.5				60	3.1
3	1.0				70	3.7
4	1.5				80	5.5
5	2.0				80	4.9
6	2.5				70	4.1
7	3.0				60	3.3
8	3.5				60	3.1
9	1.5	0.2			80	2.0
10	1.5	0.4			90	6.3
11	1.5	0.6			70	4.5
12	1.5	0.8			70	3.3
13	2.0	0.2			70	3.4
14	2.0	0.4			60	3.0
15	1.5		0.2		70	5.8
16	1.5		0.4		70	4.0
17	1.5		0.6		60	3.2
18	1.5			0.2	80	5.5
19	1.5			0.4	70	3.1
20	1.5			0.6	60	2.2

*Data represent the mean of 10 replicates, $CD_{0.05}$: 0.69, SEd: 0.35

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E. POST COCOON TECHNOLOGY SECTION

Studies on Muga cocoon (Antheraea assamensis Helfer) cooking- Effect of alkaline buffer on cooking and reeling of muga cocoon.

Jayanta Ghose, Gulrajani M.L.,* and R. Chakravorty. Central Muga Eri Research & Training Institute, Central silk Board. Lahdoigarh, Jorhat (Assam). * Department of Textile Technology, Indian Institute of Technology, Hauz-Khas, New Delhi-110 016.

ABSTRACT

Cooking of muga cocoon *(Antheraea assamensis Helfer)* with alkaline buffer has been studied with different concentration and time of treatment. In few buffer treatments, 5-7% higher silk recovery were recorded than the conventional soda cooking process. Shell weight loss and silk yarn recovery are influenced by buffer concentration and duration of treatment.

Key words : *Antheraea assamensis Helfer,* Cooking, buffer, degummimg, silk recovery.

INTRODUCTION

Muga cocoon cooking is done in alkaline solution as muga sericin is not soluble in hot water like mulberry cocoon sericin. Traditionally, locally made mild alkali solution, popularly known as 'khar' is used for cooking muga cocoon for reeling purpose. In commercial reeling, soda ash is used for cooking and silk recovery obtained was reported up to 47% (Pal *et. al,* 2005). Raw silk recovery depends on quality of the cocoon, the cooking process and efficiency of the reeling machine. Fresh cocoons having compact shell within the range of 0.45-0.55g obtained from commercial spring and autumn seasons give the highest silk recovery (Ghose *et al*, 1993). Cooking is a partial degumming process and it facilitates softening of gummy component of the cocoon shell for easy unwinding of silk filament. The degumming is generally carried out by treating with alkali, enzymes, with water and also with organic acids (Chopra *et al*, 1994). Alkali degumming is comparatively better in respect of total cost, productivity and efficiency (Chopra*et.al* ,1993). pH of the degumming bath is an important criteria for effective degumming and the pH should be maintained within the range 9.5 to 10.5 in alkaline degumming process (Mosher, 1930). As buffer system can maintain the desired pH in the cooking bath in a better way than the individual alkali, cooking of muga cocoon have been tried with an alkaline buffer (Gulrajani *et. al,* 1990). The aim of the study is to optimize the cooking process of muga cocoon to achieve higher silk recovery during reeling process.

MATERIALS AND METHODS

Materials

Cocoon : Stifled and sorted muga cocoon of autumn crop (September-October,2006) were taken for the study. The specification of the cocoon lot are given below:

Source : Central Muga Eri Research & Training Institute's experimental field, Lahdoigarh, Assam, India.
Colour : Dark grey

Shape : Oval
Voltinism : Multivoltine
Average shell weight : 0.45 g
Average filament length : 485 m
Average non-broken filament length : 235 m
Average filament fineness: 5.2 denier

Chemicals

The alkaline buffer (AR grade) of purity 99.5% was used as cooking chemicals.

Methods

Cooking : Muga cocoons were conditioned for 48 hours (27C and 65% RH). The cocoons were cooked at boiling temperature with a range of concentrations of buffer solutions. Liquor ratio was kept at 30:1. Four different time of treatment were considered. The cooked cocoons were then deflossed individually and reeled on CMER&TI muga reeling machine. The silk hanks were then conditioned and weighted. The silk waste generated during the reeling process were collected, conditioned and weighted. The conventional soda cooking was considered as control treatment. 3 gram sodium carbonate per liter of water was taken for cooking muga cocoon as control study for four different temperatures as in other treatments. pH of the solution for control treatment was recorded 10.5 – 11.1.

Evaluation of properties

Shell weight loss

The shell weight loss (%) was calculated as:
Shell weight loss(%) =[$(W_1-W_2)/ W_1]x100$, where
W_1 = weight of cocoon shell before cooking
W_2 = weight of cocoon shell after cooking

Silk recovery

The raw silk recovery was calculated as per the following relations:
Silk recovery (%) = [A/(A+B)] X 100 where
A = Weight of reelable filament
B= Weight of total silk waste generated during reeling.

4. Results and discussions

Silk recovery and Shell weight loss

From the experiment It was recorded that the recovery of silk remains in a higher range (51% and more) within a certain range of concentration and time of treatment. Around 5 – 7% increase in raw silk recovery can be obtained using this buffer as cooking media in selected concentration and time of treatment compared to the conventional soda cooking process which was taken as control treatment. The higher silk yield is attributed to uniform and optimum softening and swelling of silk gum and its dissolution during cooking process. The different concentrations of buffer solution used as well as different time of treatment for cooking muga cocoon on raw silk recovery is statistically significant and is highly significant over control (Table 1)
Further, when we compare the silk recovery with shell weight loss of cocoon, it gives an interesting result which shows higher shell weight loss does not

produce more silk. It is apparent that higher silk recovery is attributed to better swelling and softening of cocoon in every layer of cocoon shell. In higher concentration and treatment time, loss of sericin is high which ultimately reduces the silk yield. The uniform degree of swelling and softening of sericin is the prime factor for easy unwinding of filament. At much lower concentration, shell weight loss is 7.2–7.9 percent, but the yarn recovery recorded below 51 percent. The reason may be non dissolution of sericin in the inner layer of cocoon. At higher concentration, the dissolution of sericin is more and therefore silk recovery also becomes less. Shell weight loss is statistically significant on concentration and time of treatment of cooking process and it steadily increases with increase in concentration and time of treatment. (Table 2)

The increase in raw silk recovery in the buffer cooking system reduces the raw material cost in the muga silk production process. Due to higher silk yield, the cocoon requirement in the buffer process of cooking is lesser by 647 numbers thereby reducing the process cost by three hundred and fifty six rupees per kilogram conversion of muga raw silk (Table 3).

Correlation between silk recovery and shell weight loss

The regression analysis was carried out with silk recovery as the independent variable and shell weight loss as dependent variable. The correlation is found significant at 0.01 level. This is due to increase in silk recovery with increase in shell weight loss up to a certain limit. After that point, more loss in shell weight decreases the silk recovery as the gum attached with the yarn also dissolves in these conditions. Therefore, higher loss in shell weight decreases the silk yield. The regression equation and the graph showing variation in silk recovery with change in shell weight loss is depicted in **Fig. 1**

		VAR00001	VAR00002
Pearson Correlation	VAR00001	1.000	-.432**
	VAR00002	-.432**	1.000
Sig. (2-tailed)	VAR00001	.	.000
	VAR00002	.000	.
N	VAR00001	72	72
	VAR00002	72	72

**. Correlation is significant at the 0.01 level (2-tailed)

Fig. 1

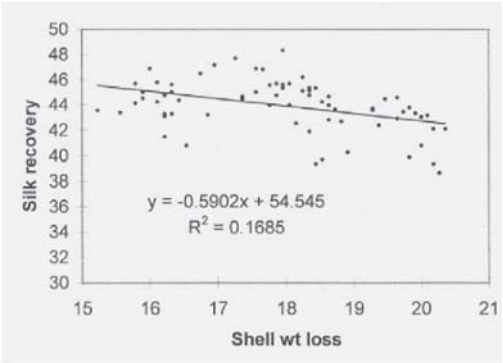

Table 1: **Effect of different concentration and treatment time on silk recovery of muga cocoon**

Conc(C) / Treatment time (T)	C1	C2	C3	C4	C5	Control	Mean
T1	47.73	51.50	52.50	50.33	50.57	44.50	49.54
	(43.70)	(45.86)	(46.43)	(45.19)	(45.38)	(41.84)	(44.73)
T2	50.33	54.60	51.27	49.43	48.73	47.13	50.25
	(45.19)	(47.64)	(45.73)	(44.68)	(44.27)	(43.36)	(45.14)
T3	48.30	51.17	48.83	47.27	45.87	47.03	48.08
	(44.03)	(45.67)	(44.33)	(43.43)	(42.63)	(43.30)	(43.90)
T4	47.53	49.27	45.50	40.93	40.10	44.17	44.58
	(43.59)	(44.58)	(42.42)	(39.78)	(39.29)	(41.65)	(41.88)
Mean	48.48	51.63	49.53	46.99	46.34	45.71	
	(44.13)	(45.94)	(44.73)	(43.27)	(42.89)	(42.54)	

	Treatment time (T)	Concentration (C)	T X C
SEd	0.22	0.27	0.53
Cd – 5%	0.44	0.54	1.07

Table 2: **Effect of different concentration and treatment time on shell weight loss of muga cocoon**

Conc(C) / Treatment time (T)	C1	C2	C3	C4	C5	Control	Mean
T1	7.17	7.63	8.23	9.23	9.73	8.10	8.35
	(15.53)	(16.04)	(16.67)	(17.69)	(18.18)	(16.53)	(16.77)
T2	7.67	9.13	9.73	10.07	11.10	11.33	9.84
	(16.07)	(17.59)	(18.18)	(18.50)	(19.46)	(19.67)	(18.25)
T3	7.67	9.23	9.87	10.30	11.50	11.53	10.02
	(16.07)	(17.69)	(18.31)	(18.72)	(19.82)	(19.85)	(18.41)
T4	7.90	9.27	9.93	10.20	11.80	11.73	10.14
	(16.32)	(17.72)	(18.37)	(18.62)	(20.09)	(20.03)	(18.53)
Mean	7.60	8.82	9.44	9.95	11.03	10.68	
	(16.00)	(17.26)	(17.88)	(18.380	(19.39)	(19.02)	

	Treatment time (T)	Concentration (C)	T X C
SEd	0.09	0.11	0.22
Cd – 5%	0.18	0.22	0.44

(Figures within parentheses are angular transformed values)

Table 3 : Comparative financial gain between conventional and improved cooking Process (Per Kg conversion of silk)

Average shell weight of cocoon (g)	Raw silk recovery (%)	Muga cocoon required to produce one Kg silk (No)	Cost of cocoon to produce one Kg silk (Rs)	Amount saved in the new process (Rs)
Conventional cooking process (Soda cooking)				
0.45	47.1	4717	2594.00	
Improved cooking process				
0.45	54.6	4070	2238.00	356.00

(Cost of cocoon = Rs. 550.00 per 1000 numbers)

Conclusion

The buffer system controls the pH in the cooking bath and therefore dissolution of sericin and other inorganic matters in the cocoon shell becomes optimum and uniform. The alkaline buffer used in this process of cooking produces 7.5% higher silk recovery than the soda cooking process. Higher pH in conventional soda cooking (10.5-11.1) dissolves more sericin in the outer layer of cocoon shell and the dissolution of silk gum in the inner layers is less which results lesser silk recovery. The buffer cooking can be taken as a viable substitute for soda cooking in the commercial muga reeling process.

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A Study of reason for Breaks occurring during Winding Process of Indian Raw Silk.

Shillin Sangappa, Subrata Roy, B.G. Patil, K.N.Mahesh* and Vineet Kumar. Central Silk Technological Research Institute, Karnataka Bengalooru-560 068, India. *Raw Silk Testing Centre, CSTRI, Sidlaghatta, Karnataka. India.E.M.U, CSR&TI, CSB, Mysore.**

ABSTRACT

In achieving the target quality and productivity of raw silk, cocoon quality alone plays a pivotal role. Certain quality of raw silk has direct bearing on surface quality of the fabric, while other on productivity level. The overall grade of Indian filature silk suffers because of low winding quality. Winding breaks is one of the crucial factor, which determines the productivity of fabric. Hence a detailed investigation has been made by the authors to locate the actual reasons for higher winding breaks of raw silk produced in Indian multiend filatures. During the time of winding, broken threads have been carefully collected for scanning electron microscope study. From the Scanning Electron Microscope photos it is revealed that the thin ends are the main cause of higher winding breaks. In case of the Indian multivoltine cocoons the size variation from upper layer to lower layer is quite high. Therefore the reeler has to be careful during casting of cocoons to achieve suitable mixing of upper layer, middle layer and inner layer of cocoons to achieve uniform size. The filament denier of inner layer of the cocoons are very thin and if all the thin layer cocoons are fed in any end, the breaking load of that particular length would be very low and is susceptible to break during winding operation. The study reveals that to improve the winding quality of raw silk reeler has to focus on suitable mixing of cocoons of different layers having different denier with utmost care.

Key words: Winding breaks, raw silk, scanning electron microscope, grading, thin end.

INTRODUCTION

The quality and productivity of raw silk depend mainly on quality of raw material i.e. cocoons, reeling process parameters, human skill and quality of water used in reeling. In achieving the target quality and productivity of raw silk, cocoon quality alone plays a pivotal role. In order to realize the best out of the raw material (cocoon), the role of other said factors are also important (Lakshmipathaiah *et al,* 2002). In the recent years, considerable development in sericulture industry has been taken place in India (Sonwalkar, 1983 & 1988). India is second largest producer of raw silk across the globe. Raw silk is the yarn produced from several numbers of cocoon and which depends on targeted denier. In India the multiend filatures are consistently producing “A grade to 2A grade” raw silk from multivoltine and bivoltine cocoons respectively (Anon, 2003). There are eleven commercial characteristics of raw silk as per international grading of raw silk and the grade of raw silk is determined as per the said eleven commercial quality parameters. Each commercial quality has definite impact either in quality or productivity of final fabric. Certain quality of raw silk has direct bearing on surface quality of the

fabric, while other on productivity level. The overall grade of Indian filature silk suffers because of low winding quality. This commercial character is one of the crucial attributes which determinesthe productivity of fabric. High winding breaks enhance down time in each operation in preparatory and weaving resulting higher cost of production due to high wastage and low productivity. The winding breaks of the raw silk are mainly due to heavy gum spots, cleanness defects, improper knotting, loose ends, bad handling and thin ends (Keizo Yajima, 1998). It is generally believed that size deviation is having direct bearing on winding breaks. The study (Shillin Sangappa *et al,* 2008) reveals that there is no correlation between winding breaks and size deviation. Control inspection study at Silk Conditioning and Testing House, Central Silk Board, Bangalore, India confirms that overall grade of Indian multiend filature raw silk mainly suffers from higher winding breaks as compared to Chinese raw silk and as a result power loom industry in India prefers Chinese silk for their fabric production. This is one of the main reasons India imports considerable quantum of raw silk from China for power loom silk industry.

Hence a detailed investigation has been made by the authors to locate the actual reasons for higher winding breaks of raw silk produced in Indian multiend filatures to counteract the problem.

MATERIALS AND METHODS

To conduct the study 200 kilogram of cocoons have been reeled batch wise in a private multiend reeling unit at Sidlagatta reeling cluster, Karnataka. Adequate care has been taken in designing of the experiment to minimize main variable factors, like gum spots, cleanness defects, knotting, bad handling etc., which affects the winding breaks. Eight cocoons per end have been reeled at a time with a take up speed of 100 meters per minute. The small reels were permeated in reel vacuum reel permeation chamber at a pressure 300 Hg for 3 cycles and then taken for re-reeling to make standard hank of 1.5 meters weighing 70 grams. The re-reeling temperature have been maintained at 37 to 40oC to expel the water to achieve optimum dry age. The dried skeins have been laced with standard cotton thread at six different place and taken out from the reels and conditioned as per the standard methods.

For winding study 40 skeins have been mounted on the swift of winding machine carefully. After removal of the lacing threads carefully the winding test have been conducted. The 40 skeins are totally wounded and breaks noted down hour wise. During the time of winding breaks the threads are carefully collected for scanning electron microscope study.

The mounted specimens have been coated with 20 nm thickness of gold on their surface, in a Sputter Coater (EMS-550) to minimize charging under the electron beam. The gold coated samples are observed using scanning electron microscope (SEM) (JEOL 100 CX II ASID 4D, Tokyo Ltd., Japan) under an accelerating voltage of 20 kV with a beam current 0.1 nA. The photographs are taken at 500 and 1000 magnification for observation.

RESULTS AND DISCUSSION

From the Scanning Electron Microscope photos (Figure 1, 2, 3, 4 and 5) it is revealed that the thin ends are the main cause of higher winding breaks.

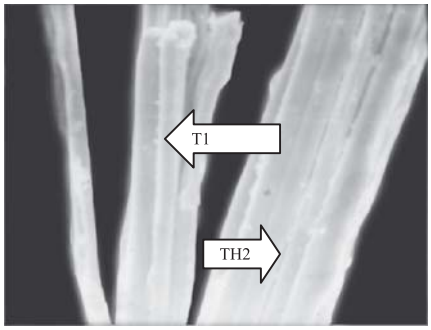

Fig 1: Broken end of raw silk occurred during winding operation (T1) reveals thin end compared non-broken part (TH2) (x500)

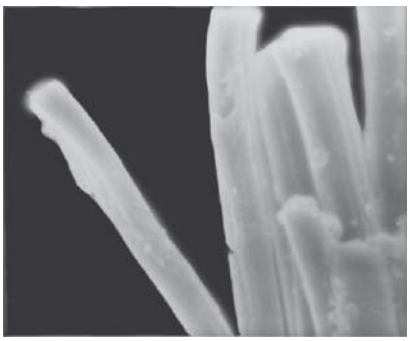

Fig 2: Magnified view of broken end of raw silk occurred during winding operation (x1000)

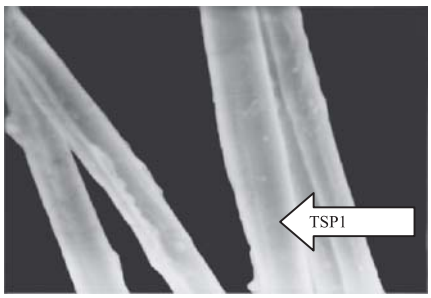

Fig 3: Magnified view of broken end splitted part (TSP1) (arrow head) and clearly shows last layer of cocoon filaments. (x1000)

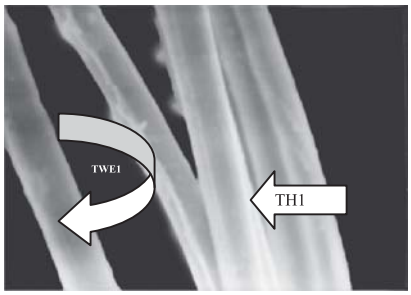

Fig 4: Broken end of raw silk (TH1) reveals weak cocoon filament (TWE1) also causes breaks during winding operation (x1000)

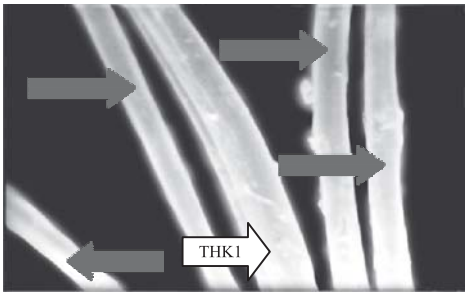

Fig 5: Magnified view of thin ends (brown arrows) and also only one Thicker end (THK1) (x500).

The filament denier of the mulberry cocoons decreases over the length significantly. The variation of the filament denier is shown in Figure 6.

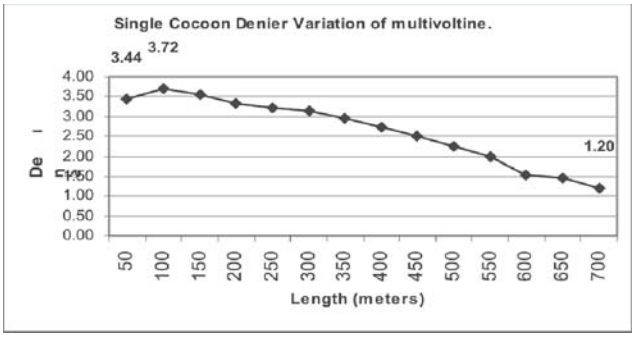

Fig. 6 Average filament denier variation in Multivoltine cocoons.

In case of the Indian multivoltine cocoons the size variation from upper layer to lower layer is quite high. Figure 6 indicates that filament denier in upper layer of cocoon is 3.72 and last layer 1.2 respectively, the average standard deviation is 0.84. Therefore the reeler has to be careful during casting of

cocoons to achieve suitable mixing of upper layer, middle layer and inner layer of cocoons to achieve uniform size. Any mistake in mixing of cocoons during the time of reeling would create un uniformity in size (denier). As the inner layer of the cocoons are very thin and if majority of last layer cocoons are fed in any unit time the breaking load of that particular length would be very low. Technically this would create thin filament which are susceptible to break during winding operation due to not withstanding winding tension during winding operation. SEM study has clearly shown that all the breaks occurred due to thin ends. The pattern of breaks during each hour is given in Figure 7.

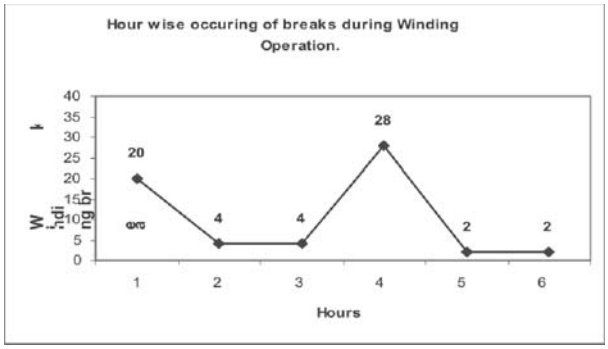

Fig. 7 Occurrence of breaks per hour.

As the winding time is the reverse timing of actual reeling operation, it is interesting to note that during last hour of reeling and just before lunch time winding breaks were abnormally high as compared to 2, 3 5, and 6 th hour. This phenomenon supports the observation from the SEM study too. During the lunch time and last hour of reeling reelers try to consume all the cocoons resulting imbalance in mixing of different layers of cocoons which intern produces thin end and hence impair the winding quality. Hence the study could locate main reasons of winding breaks of raw silk reeled on Indian multiend filatures.

CONCLUSIONS.

The study reveals that to improve the winding quality of raw silk reeler has to focus on mixing of cocoons with utmost care. If he fails to maintain the particular no of cocoons with proper mixing of different layer of cocoons winding quality cannot be improved as per the international standard.

ACKNOWLEDGEMENT

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Draping Behavior of Silk and Naturally Color Linted Cotton Union Fabrics.

Dr. Sadhana D. Kulloli and Dr. Shailaja D. Naik. Department of Textiles & Apparel Designing, College of Rural Home Science, UAS, Dharwad – 580 005.

Abstract

Two varieties of Naturally brown coloured cotton viz.,. Dharwad Desi Colour Cotton – 1 (DDCC-1) and Dharwad Brown Hirsutum – 250 (DBH-250) handspun yarns were shot with Muga, Mulberry and Tasar silks to produce eco-friendly union fabrics. Totally six union and three control fabrics were produced to know the draping behaviour. Results revealed that the weft way bending length and the warp way crease recovery of all the union fabrics was higher than their warp way bending length and weft way crease recovery respectively because the DDCC-1 and DBH-250 weft yarns are coarser, heavier and sized. The starch makeshe yarns hard thus making the fabric stiffer, thicker and heavier. Irrespective of warp way and weft way crease recovery, Muga control fabric (MU, 105.15^0) and Muga X DBH-250 (MUD_2, 102.30^0) followed by Muga X DDCC-1 (MUD_1, 100.75^0) union fabrics showed highest cloth recovery. Among the control samples, Tasar (TA) draped in 5 nodes, Muga (MU) with 4 nodes and Mulberry (MB) 3 nodes while among union fabrics Muga X DDCC-1 (MUD_1) and Tasar X DBH-250 (TAD_2) draped in graceful 6 nodes followed by Muga X DBH-250 (MUD_2) and Tasar X DDCC-1 (TAD_1) in 5 nodes indicating these fabrics draped better than the control fabrics. The co-efficient of determination (R^2) values revealed that the influence of cloth stiffness and crease recovery on drapability was 92.6% (warp way) and 91.0% (weft way) among Muga and union fabrics, 79.0% (warp way) and 74.5% (weft way) among Mulberry and union fabrics and 93.9% (warp way) and 92.6% 9weft way) among Tasar and union fabrics. Thus, these union fabrics are new and unique of its kind and are not only suitable for dress materials but also for furnishings. However, the hand spun DDCC-1 and DBH-250 yarns showed unequal distribution of slubs and snarls all along its length which is an added advantage of fancy appearance and texture. Therefore, it is boon for the cotton cultivators to grow colour cotton on commercial scale, the local spinners to earn livelihood and enhance the socio-economic status.

Effect of Sizing Agent (Gum Acasia) on Silk.

Dr. (Mrs.) Jyoti V. Vastrad[1]*, Dr. Sannapapamma K.J.[2] and Mr. U.C. Javali[3]. 1*: Associate Professor and Corresponding Author, 2: Assistant Professor, Department of Textiles and Apparel Designing, College of Rural Home Science, University of Agricultural Sciences, Dharwad 580 005, Karnataka, India. 3 : Scientist, CSTRI, Central Silk Board, Madivala, Bangalore – 500 068, Karnataka, India.

Abstract

There is an increasing popularity for the use of silk made-ups and garments across cross-section of the society. Although relatively durable, silk is a protein fibre similar to human hair and hence requires special care to maintain its appearance. Dry cleaning helps maintaining the hand and feel and sheen of silks. The natural coating on silk fibre responds well to warm water, hence has the advantage of refreshing silk and giving it a better drape during hand washing. Silks tend to sag and loose their stiffness in spite of any amount of care taken during laundering. Hence, sizing the laundered silks with a mild sizing agent enhances the hand and feel of silks fabrics. The oldest and the best known sizing agent for silk is gum arabic, also known as turkey gum, gum senegal and so on. Gum arabic gives body in finishing silk and rayon fabric without loss of transparency hence aids in maintaining the aesthetic appearance of the fabric. Therefore the present study was undertaken with the objective to know the effect of varied concentrations of gum arabic on silk fabric. The influence of crease recovery and bending length on draping behaviour of sized silks of different weights was also studied. Sizing enhances the weight of the samples. Absorption varies with the weight of silks and also the cloth density. Decreasing trend in the cloth recovery is observed. However, stiffness and the recovery have an inverse relation and the results exhibited were highly significant due to the significant variations in the recovery of the warp and weft with varied size concentrations. Bending length was directly related to weight of the fabric and the size concentrations and hence showed significant results. Drape coefficient values of the samples increased with the increase in size concentration. Cloth crease recovery was negatively related to drape and was significant for the warp direction. Cloth stiffness significantly affected the drape parameters.

Aesthetics of Ahimsa silk union made-ups.

Dr.(Mrs.) Sannapapamma K. J. Assistant Professor, Department of Textiles and Apparel Designing, College of Rural Home Science, UAS, Dharwad – 5. Karnataka, India and Dr. (Mrs.) Shailaja D. Naik, Professor and Head.

ABSTRACT

Karnataka is the premier mulberry silk producing state in India, contributing nearly 60 per sent of the country's total production. Around 764 drainages distributed in different parts of Karnataka caters to the seed requirement of the Seri culturist. During drainages operation moths emerges out by piercing the cocoons thus become unreelable. Such cocoons amount to about 240 Tons per year, hence proper utilization of these coons is of utmost importance for product diversification. The silk extracted from mulberry pierced cocoons letting moth to emerges out by piercing the cocoon resulting into unreelable state is often referred of **"Ahimsa Silk".** Ahimsa silk spin from CSTRI machine has fine count with greater tenacity and elongation, aptly suitable for handloom sector because of its unique physical features that can be fabricated into beautiful fancy textile made-ups, further used as dress materials and shirtings. The effects of fabric structure and mechanical properties on the drape characteristics of Ahimsa silk union made-ups were studied. Keeping Ahimsa silk union as weft, the dress materials were woven in cotton, art silk and filature silk, while the shirtings were woven in cotton, tricot and filature silk on pit looms. These made-ups were subjected for drapability test to assess the aesthetic property. Drape of any fabric is expressed in terms of drape coefficient (%) and the number of nodes formed when draped. The drape coefficient of a fabric is directly related to cloth density, cloth sett and relative firmness of the weave. Among the test samples the made-ups comprised of cent percent silk, which were found to be flexible and pliable than the other union made-ups.

Key words – CSTRI Machine, Ahimsa Silk, Bending length, Crease recovery, Drapability

Studies on Characteristics of Woven Fabrics containing Core Spun Tasar Silk Yarn in Weft.

N.S. Gahlot*, Z.M.S. Khan, N.G. Ojha and N. Suryanarayana, Central Tasar Research and Training Institute, Central Silk Board, P.O. Piska-Nagri, Ranchi - 835 303, India.**

ABSTRACT

The properties of plain woven fabrics made of core spun tasar silk yarn in weft having tasar silk fibres (silk wastes) as covered materials with cotton; jute and polyester yarn as core material and tasar reeled in warp for all the fabric samples have been studied. Three different types of core spun weft yarn namely; tasar/cotton (7.0 g/tex), tasar/jute (5.3 g/tex) and tasar/polyester (28.0 g/tex) were prepared on Amber Charkha. The core spun tasar silk fabrics constructed on frame loom in the institute have been investigated and the physical properties of core spun fabrics (single reeled x core spun yarn) were compared with the tasar silk fabrics containing tasar reeled in warp and tasar spun yarn (2.7 g/tex) in weft. The physical characteristics of these three varieties of core spun fabrics were correlated with thread density, yarn count, fabric weight, tensile strength, breaking elongation, tearing strength, abrasion resistance, crease recovery angle and flexural rigidity. It is observed that the tensile strength, abrasion resistance and flexural rigidity of the fabric made from core spun tasar silk yarn in weft which is higher compared to that of tasar silk fabric (tasar reeled x tasar spun yarn) or control and crease recovery angle is closer to control. The fabric made out of core spun tasar yarn in weft is thicker, durable, dull lusture and softer; can be used for furnishing fabrics, dress materials, ready made garments, carpets followed by ladies wear. The core spinning process by tasar silk can be used for utilization of tasar wastes and it can be reduced the use of tasar silk in the fabric without affecting the lusture and maintain demand of wild silk.

KEY WORDS:

Core spun yarn, Cotton yarn, Jute yarn, Polyester yarn, Physical properties, Tasar fabrics.

1 INTRODUCTION

The tasar silk industry is a cottage based rural industry in India and about 50-60% of the yarn is produced through reeling process and rest of the quantity remains as silk waste by which spun yarns can be prepared through waste silk spinning [1, 2]. The tasar fibre is composed of minute filaments or fibrils. It is irregular course and brown in colour but has an advantage of unusual strength and durability over mulberry silk. The tasar silk fabrics are mostly used in the form of thicker varieties of fabrics made from spun yarn namely tasar Ghicha, tasar-Ghicha-noil and tasar Katia yarn mostly catering to the export markets The main varieties of tasar silk goods exported are dress materials, ready made garments, carpets, furnishing fabrics followed by Sarees [3]. The fabric characteristics mainly depend on the structure and type of yarn used. The development of new yarn structures raises the question about the nature and quality of fabrics made out of it. It has been studied [4, 5] that different types of yarn making processes not only produce different yarn structures, but the differences are reflected in the performance of fabrics

made from them. The construction oftasar core spun yarn and its properties has been studied. Earlier research [6, 7, 8 and 9] has shown that core spun tasar silk yarn can be used in weft and developed for diversified tasar silk products by the core spinning at a reduced cost. But the behavior of fabrics constructed by core spun tasar silk yarn in weft and tasar reeled in warp totally unexplored. There is scarcely any study reported on this subject. Therefore, the present study was, undertaken to investigate the behavior of woven fabric made out of core spun tasar silk yarn in weft and its physical properties compare with the properties of fabric made from pure tasar spun yarn in weft.

2 MATERIAL AND METHODS

2.1 Raw Materials

Different tasar silk wastes were obtained after processing (stifling or drying, cooking of cocoons and reeling) of defective tasar cocoons or pierced cocoons were collected from various sections of Central Tasar Research and Training Institute, Ranchi. The tasar silk waste was used in staple fibre form for wrapping and covering the core material. Three types of spun yarns namely cotton; jute and polyester were used as a core in weft (Table 1)

2.2 Methods

After preliminary cleaning, the degumming of silk waste was carried out by using Soda for 1.7 kg of material. 16 liters of water 510 gm of soda boiled for 50-60 minutes. After this process, the material was boiled for 5-10 min. in plain water further the material was thoroughly washed in plain water for removing the gum and foreign materials. The degummed tasar waste may be dried at room temperature or in Sunlight, and thenit was opened by hand for cleaning. The soft opened silk wastes were passed through coarser fillet and finer Fillet. The lap received from finer fillet is cut into fibre of 1” to 1.25” length and finally the materials were used for spinning followed by drawing. Core spinning process was carried out by utilizing tasar silk waste through Amber Charkha using cotton, jute and polyester yarn as core material and wrapping them with tasar silk fibres. All the three fabric samples were produced on frame loom using three types of core spun tasar silk yarns in weft. Controlled fabric was obtained from yarns spun purely with tasar fibres using the above machines.

3 RESULTS AND DISCUSSION

3.1 Tensile Properties

Tensile properties of warp and weft yarns, taken out from core spun tasar fabrics, (Fabric A, B and C) are given in the Table 1. As the warp yarn is same in all the three fabrics,the average tensile properties from three fabrics are taken. Weft yarn of Fabric C shows maximum tenacity. Bearing in mind that the yarn with different structure were used in the weft direction, so that the weft - wise fabric strength would be roughly in proportion to yarn strength. Also, the parallel laying of fibres in weft yarn along the load direction results in uniform and maximum load sharing by all the component fibres in weft direction of the fabric[5]. With core spun in weft (Fabric C), the tensile strength in weft direction is higher (Table 2). The braking elongation of weft yarn from Fabric C is low, the braking elongation of fabrics in weft direction shows completely different trend.

3.2 Tearing Strength

It is clear from Table 2 that the weft tear of Fabric C is higher than that of Fabric A and B, but lower than control sample and similar trend was obtained in warp direction. The tear strength is mainly depends on the yarn strength, fabric structure and surface characteristics of yarn. A tight fabric allows only one yarn to break at a time as the tear propagates. A loose fabric allows more yarns to carry the load at any one time [5].

Table 1 - Fabric construction details

[Warp: Single reeled tasar yarn 60 denier or 88.58 tex]

Fabric sample	Weft composition		Fabric set		Fabric weight g/m^2	Tenacity (g/tex)
	Yarn type (Cover/core)	Yarn count tex	Ends/inch	Picks/inch		
Fabric A	Tasar/Cotton	16.97	60	54	175	7.0
Fabric B	Tasar/Jute	8.51	60	44	198.4	5.3
Fabric C	Tasar/Polyester	72.02	60	63	115.6	28.0
Control	Tasar spun	12.92	60	47	125.2	2.7

Table 2 - Characteristics of core spun tasar fabrics

Fabric sample	Tensile strength (N)		Breaking elongation (%)		Tearing strength (g)		Flexural rigidity (mg-cm)		Crease recovery angle (deg.)		Abrasion resistance (Strokes)
	Warp	Weft	Warp	Weft	Warp	Weft	Warp	Weft	Warp	Weft	3017
Fabric A	164.5	389.7	31	15	1446	3635	71.7	384.2	94	90	2217
Fabric B	133.2	369.6	30	10	1498	--	34.3	1088.7	89	60	1933
Fabric C	160.1	406.3	26	21	1600	4006	107.1	281.9	89	97	1717
Control	160.4	298.6	33	20	1958	4454	115.9	373.0	91	99	1467

3.3 Flexural Rigidity

Table 2 shows that the result would seem to be odd in warp and weft flexural rigidity. The weft flexural rigidity of Fabric A is higher that of Fabric C. The maximum weft flexural rigidity of fabric B is due to the compact weft yarn structure as compared to fabrics A and C.

3.4 Crease Recovery

It is evident from Table 2 that the weft crease recovery of Fabrics C is little higher than that of Fabric A and B, but nearest to control sample. The reason may be that the bulky weft yarns of Fabric C cause more contact area that results in less inte- yarn movement during creasing. The axial alignment of fibres in weft yarn of Fabric C results in further increase in crease recovery.

3.5 Abrasion Resistance

Abrasion resistance of Fabric A is higher than that of Fabric B, C and Control sample. As in all the fabrics the warps are same, the difference in abrasion resistance is mainly due to the variation in weft yarn structures.

4 CONCLUSIONS

4.1 Fabric samples made out of core spun tasar yarn in weft shows that the tensile strength in weft direction is higher as compare to control sample. A similar trend is also observed in abrasion resistance. The weft crease recovery of fabric is nearer to control sample.

4.2 With the increase tensile strength in weft wise, the breaking elongation reverses for all the samples.

4.3 Tear strength of core spun fabrics is lower than control sample and weft flexural rigidity shows no specific trend. Core spun tasar yarn in weft does not have any significant effect on crease recovery angle.

4.4 The warp-wise properties in terms of tensile strength, tear strength and crease recovery are affected by making substitution in the type of weft yarn. Slightly less the warp-wise flexural rigidity is observed as compare to control.

4.5 Fabric made out of core spun tasar yarn in weft which is thicker, durable, dull lusture and softer; can be used for furnishing fabrics, dress materials, ready made garments, carpets followed by ladies wear. The core spinning process by tasar silk can be used for utilization of tasar wastes and it can be reduced the use of tasar silk in the fabric without affecting the lusture and maintain demand of wild silk.

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Information Technology for Thai Silk Yarn Development.

Vorapot Raksang. Queen Sirikit Sericulture Center (Nakhorn Ratchasima), Thailand, 1887 Mittraparp Rd., Amphur Muang ,Nakon ratchasima 30000.

Abstract

This research aimed to use Information Technology for development silk yarn in Thailand. Yellow silk yarn produced by farmer in northeastern part of country was randomly collected. And the locations where those were collected would be noted by using Global Positioning System (GPS). Test qualities of silk yarn in average size, tenacity, elongation , skein length, skein weight, visual inspection in Thai silk standard method that establish in our country as 1). Defect of waste slug knot, 2). Cohesion, 3). Evenness in size, 4). Uniformity of colour and 5). Cleanness .

Thai raw silk (yellow silk yarn) of 520 samples had been randomly collected since 2005 to 2007 from 13 provinces as Nakhon ratchasima, Buri ram, Surin, Srisaket, Ubon ratchathani, Nhongkhai, Udon thani, Kalasil, Khon kaen, Amnat chareon, Roi et, Maha sarakham and Nong bua lamphu and theirs GPS points. Qualities of Thai yellow raw silk were tested. The silk yarn qualities data and GPS point were input to digital maps. For examples the location between latitudes 15°47' N to 14°7' N and longitudes 101°11' E to 103°1' E was Nakhon ratchasima province. And the qualities test from 91 samples were skein length as172 $\pm$15 cm., skein weight as 108$\pm$25 g, average size as 298$\pm$69 denier, tenacity 3.2$\pm$0.3 g/d, and elongation as 22.8$\pm$3.6 %. For visual inspection, the average 100 line found evenness in size as 0.97$\pm$1.64 line, waste, slug, knot 1.08$\pm$0.9 line, cohesion 31.6$\pm$22 line, uniformity of colour 0.2$\pm$0.92 line and cleanness 0.03$\pm$0.11 line. Whereas, at the lattitudes 15° 8' N to 14°48' N and the longitudes 102°18' E to 102°34' E was Amphur Chakkra rat in Nakhon ratchasima province. The qualities test from 12 samples were skein length as 163 $\pm$15 cm., skein weight as 111$\pm$21 g, average size 291$\pm$65 denier, tenacity 3.1$\pm$0.1 g/d, and elongation 26.4$\pm$ 2.9 %. For visual inspection: average 100 line found evenness in size 0.42$\pm$0.48 line, waste, slug, knot 0.36$\pm$0.3 line, cohesion 33.9$\pm$23 line, uniformity of colour 0.01$\pm$0.03 line and cleanness 0.01$\pm$0.03 line.

This information was useful for Thai silk yarn quality development, information for silk yarn consumer (weaves).

Keywords: silk yarn quality, information technology, GPS, Thai silk standard

Introduction

Thailand is located on middle of Indo-china peninsula, between latitude 5°27' N to 20°27' N and longitude 97°22' E to 105°37' E . The area is occupied about 513,115 square kilometers. The areas which are used for agricultural land 41 % such as rice 25%, field crops 12%, fruit and perennial 8%.

Sericulture in Thailand is broadly dispersed, because of properly climate and regularly income. However, the career of sericulture is able to reduce labour migration and it is environmental friendly.

The qualities of Thai silk yarn are variable depending on locations and culture of farmers such as skein length, size.

This research aimed to use Information Technology for development silk yarn in Thailand, by collecting Thai silk yarn (yellow silk yarn) production from

various locations in the northeastern of Thailand (main location) and theirs qualities were determined.

This information is useful for Thai silk yarn quality development, and information for silk yarn consumer (weaver) for develop their fabric quality and productions.

Materials and Methods

1. Sampling collected Thai silk yarn from farmer in the northeastern of Thailand including theirs GPS marks.

2. Quality test by visual inspection using Thai silk standard method that established in the country in 2005 as followings: 1) defect of waste slug knot 2) cohesion 3) evenness in size 4) uniformity of colour 5) cleanness. By use 5x10 cm black paper insert under 100 line of yarn and count abnormal properties of 5 qualities above one line equal one point, do the same 10 times and used average data.

3. Other qualities test of silk yarn as: in average size, tenacity, elongation, skein length, skein weight.

4. All information (qualities and locations) was evaluated by using digital mapping.

Results and Discussion

Sampling collected since 2005 to 2007, Thai raw silk of 520 samples were randomly collected from 13 provinces as Nakhon ratchasima, Buri ram, Surin, Srisaket, Ubon ratchathani, Nhongkhai, Udorn thani, , Kalasil, Khon kaen, Amnat chareon, Roi et, Maha sarakham and Nong bua lamphu and theirs GPS points. Qualities of Thai yellow raw silk are tested.

The results were shown as the followings :

- Nakhon ratchasima province qualities test from 91 samples were skein length as 172 ±15 cm. Skein weight 108±25 g, average size 298±69 denier, tenacity 3.2±0.3 g/d, elongation 22.8±3.6 % ,for visual inspection: average 100 line found evenness in size 0.97±1.64 line, waste,slug,knot 1.08±0.9 line, cohesion 31.6±22 line , uniformity of colour 0.2±0.92 line and cleanness 0.03±0.11 line .
- Buri ram province qualities test from 33 samples are skein length is 164 ±38 cm. Skein weight 22±23 g ,average size 228±58 denier, tenacity 3.0±0.2 g/d, elongation 23.0±5.2 % ,for visual inspection: average 100 line found evenness in size 2.22±3.64 line, waste,slug,knot 1.02±0.6 line, cohesion 15.8±24 line , uniformity of colour 0.2±0.80 line and cleanness 0.05±0.09 line .
- Surin province qualities test from 113 samples are skein length is 179 ±39 cm. Skein weight 86±29 g ,average size 177±61 denier, tenacity 3.1±0.3 g/d, elongation 21.1±3.6 % ,for visual inspection: average 100 line found evenness in size 1.54±1.72 line, waste,slug,knot 0.76±.8 line, cohesion 13.5±12 line , uniformity of colour 0.2±0.37 line and cleanness 0.06±0.29 line .
- Srisaket province qualities test from 31 samples are skein length is 163 ±28 cm. Skein weight 79±16 g ,average size 287±60 denier, tenacity 3.0±0.2 g/d, elongation 21.2±3.1 % ,for visual inspection: average 100 line found evenness in size 4.52±5.34 line, waste,slug,knot 2.38±3.7 line, cohesion 18.9±14 line , uniformity of colour 0.2±5.40 line and cleanness 0.07±0.14 line .

- Ubon ratchathani province qualities test from 16 samples are skein length is 153 ±59 cm. Skein weight 92±28 g ,average size 241±55 denier, tenacity 2.8±0.2 g/d, elongation 16.9±2.9 % ,for visual inspection: average 100 line found evenness in size 4.28±4.09 line, waste,slug,knot 3.10±2.9 line, cohesion 14.9±8 line , uniformity of colour 1.5±4.10 line and cleanness 0.41±0.56 line .
- Nhongkhai province qualities test from 40 samples are skein length is 165 ±26 cm. Skein weight 103±30 g ,average size 263±72 denier, tenacity 2.9±0.3 g/d, elongation 21.4±3.9 % ,for visual inspection: average 100 line found evenness in size 1.52±2.87 line, waste,slug,knot 1.83±1.4 line, cohesion 18.6±9 line , uniformity of colour 0.1±0.12 line and cleanness 0.02±0.07 line .
- Udorn thani province qualities test from 19 samples are skein length is 156 ±9 cm. Skein weight 111±32 g ,average size 281±82 denier, tenacity 3.0±0.4 g/d, elongation 20.3±2.6 % ,for visual inspection: average 100 line found evenness in size 2.28±1.74 line, waste,slug,knot 2.73±2.4 line, cohesion 38.1±12 line , uniformity of colour 0.9±1.29 line and cleanness 0.33±1.13 line .
- Kalasil province qualities test from 12 samples are skein length is 147 ±11 cm. Skein weight 109±37 g ,average size 312±77 denier, tenacity 2.8±0.2 g/d, elongation 23.2±2.5 % ,for visual inspection: average 100 line found evenness in size 4.76±5.20 line, waste,slug,knot 1.47±1.00 line, cohesion 21.8±19 line , uniformity of colour 0.9±2.66 line and cleanness 0.48±0.51 line .
- Khon kaen province qualities test from 50 samples are skein length is 149 ±11 cm. Skein weight 113±38 g ,average size 301±81 denier, tenacity 3.0±0.2 g/d, elongation 23.4±4.1 % ,for visual inspection: average 100 line found evenness in size 1.64±1.81 line, waste,slug,knot 1.42±1.13 line, cohesion 37.4±20 line , uniformity of colour 0.5±1.33 line and cleanness 0.13±0.31 line .
- Amnat chareon province qualities test from 16 samples are skein length is 164 ±9 cm. Skein weight 81±34 g ,average size 256±79 denier, tenacity 2.9±0.9 g/d, elongation 18.2±4.1 % ,for visual inspection: average 100 line found evenness in size 1.85±2.70 line, waste,slug,knot 1.53±1.43 line, cohesion 28.7±12 line , uniformity of colour 0.2±0.26 line and cleanness 0.34±0.81 line .
- Roi et province qualities test from 42 samples are skein length is 159 ±16 cm. Skein weight 106±20 g ,average size 221±76 denier, tenacity 3.1±0.3 g/d, elongation 19.1±3.8 % ,for visual inspection: average 100 line found evenness in size 2.69±2.68 line, waste,slug,knot 2.30±1.77 line, cohesion 12.0±8 line , uniformity of colour 0.2±0.45 line and cleanness 0.09±0.15 line .
- Maha sarakham province qualities test from 43 samples are skein length is 163 ±20 cm. Skein weight 112±16 g ,average size 313±88 denier, tenacity 3.0±0.2 g/d, elongation 23.5±2.7 % ,for visual inspection: average 100 line found evenness in size 2.14±1.89 line, waste,slug,knot 1.63±1.22 line, cohesion 17.0±18 line , uniformity of colour 0.3±1.37 line and cleanness 0.03±0.05 line .
- Nong bua lamphu province qualities test from 14 samples are skein length is 152 ±10 cm. Skein weight 115±48 g ,average size 286±83

denier, for visual inspection: average 100 line found evenness in size 1.47±1.54 line, waste,slug,knot 1.27±0.40 line, cohesion 11.5±6 line , uniformity of colour 0.0±0.03 line and cleanness 0.03±0.05 line .

The silk yarn qualities data and GPS point are input to digital maps. For example between lat 15°47' N to 14°7' N and lon 101°11' E to 103°1' E is Nakhon ratchasima province qualities test from 91 samples are skein length is 172 ±15 cm. Skein weight 108±25 g ,average size 298±69 denier, tenacity 3.2±0.3 g/d, elongation 22.8±3.6 % ,for visual inspection: average 100 line found evenness in size 0.97±1.64 line, waste,slug,knot 1.08±0.9 line, cohesion 31.6±22 line , uniformity of colour 0.2±0.92 line and cleanness 0.03±0.11 line. And at lat 15° 8' N to 14°48' N and lon 102°18' E to 102°34' E is amphur Chakkra rat in Nakhon ratchasima province qualities test from 12 samples are skein length is 163 ±15 cm. Skein weight 111±21 g, average size 291±65 denier, tenacity 3.1±0.1 g/d, elongation 26.4±2.9 %, for visual inspection: average 100 line found evenness in size 0.42±0.48 line, waste, slug, knot 0.36±0.3 line, cohesion 33.9±23 line , uniformity of colour 0.01±0.03 line and cleanness 0.01±0.03 line.

Conclusion

The qualities of Thai silk yarn are various depend on location and culture of farmers such as skein length, size .

This information are useful for Thai silk yarn quality developement, information for silk yarn consumer (weaver).

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Fig. 1 Various qualities of Thai

Fig. 2 Visual inspection in Thai silk standard method

The Study on Model Farm Group for Thai Silk Production under the Community Silk Reeling Factory System.

Somying Chuprayoon[1], Suban Sopha[2], Jiralak Preedee[3]. 1.Subject Matters Specialist Level 8, Technology Promotion and Transfer Sub-division, The Queen Sirikit of Institute Sericulture. 2. Director, Subject Matter Specialist Level 8, The Queen Sirikit Center of Sericulture Roi-et. 3. Subject Matters Specialist Level 6, The Queen Sirikit Center of Sericulture Khon-kaen.

Abstract

Silk yarn production through a network of community silk reeling factory is a system that promotes the silk yarn production to acquire a uniformity of silk yarn product that meets the demands of weaving industry on both qualitative and quantitative aspects. The study on the farmer model groups for Thai silk production under the community silk yarn reeling factory network took place at farmers groups who produced cocoons for the network member factories, as well as the 3 selected community silk reeling factories to produce silk yarn that meet the national silk quality standard THAI AGRICULTURAL COMMODITY AND FOOD STANDARD TACFS 8000-2005. The selected farmers' groups were; 1) Khon Kaen Province – Ban Nonsila Community Silk Reeling Factory and 40 members, 2) Buriram Province – Ban Haisok-Nonjikpattana Community Silk Reeling Factory and 30 members, and 3) Mahasarakam Province – Ban Non-ngarm Community Silk Reeling Factory and 30 members.

All members were scheduled to raise one egg sheet each for 3 cycles of silkworms; May, July and September 2007. The average total cocoon yield per farmer was 51.04 kilograms with the average total production cost of 105.58 Baht per kilogram. The realized average total income was 5,216.32 Baht per farmer.

The community silk reeling factories bought cocoon products from farmers at an approximate volume of 480 – 600 kilogram per cycle at an average cocoon price of 101.81 Baht per kilogram. Cocoons were then reeled by small community silk reeling machines powered with less than 5 Hp. The acquired cocoons to silk yarn ratio average was 10.3 to 1 , resulting in total average silk yarn product was 54.64 kilograms for each cycle. This quantity enabled the community silk yarn factory network to commercially work with the network members. (Table 1-3). The average production cost of the community silk reeling factories was 1,264.96 Baht per kilogram, if farmers' labour cost was not calculated, the production cost would reduced by 40-50 percent. The average income from silk yarn reeling, which was 1,429.19 Baht per kilogram (Table 5-7) the factories still made an average profit of164.23 Baht per kilogram. The silk yarn derived from 3 factories was tested for silk yarn elongation (%), tenacity (gram). The results shown that all the samples had an average elongation of 23.53 percent and an average tenacity of 4.61 gram respectively (standard elongation is not less than 18% and tenacity not less than3.7gram).

INTRODUCTION

1. Rationale and Background

Being an agricultural commodity born with Thai culture, Thai silk has provided a steady income source for farmers throughout the year. In 2004, there were approximately 136,884 sericulture farmers occupying an approximate mulberry planting area of 155,510 rai (or 24,882 hectares). The total silk yarn production was 1,420 tones, of which 1,100 tones were handicraft silk and 320 were industrial silk. This total quantity was far to meet domestic demand, thus 515 tones of raw silk were imported during the year (Department of International Trade, 2004). Among all sericulture farmers, 80% live in the northeastern region. Most farmers rear silkworms for household use, with surplus sold out for disposable family incomes. The quality of silk yarn produced by these farmers varies and makes it difficult for commercial silk weaving business.

In 1999, the Department of Agricultural Extension (DOAE) together with the Development of Silk Production in the Northeast Project (DEVSILK) initiated community networks in the form of 'community silk yarn reeling factory' to be a centre for the purchase of cocoons. Farmers who produced Thai improved silkworm varieties on a commercial scale and belonged to a farmers' group under a network sold their cocoon product to the factory. The aim was to produce good quality and quantity silk yarn to feed into the weaving industry regularly. The pilot project was conducted in Khon Kaen, Mahasarakam and Buriram (DEVSILK, 2000).

The results of a monitoring study and a seminar among the community silk yarn reeling factories in 2003 revealed that the most important factor in operating the factory was the continuation of raw materials, i.e. fresh cocoons. Many farmers' groups still lacked of farm management that ensured high cocoon yield, (Suwit, 2003). This directly affected the factories' operations which resulted in high production cost for silk yarn. Small volume of silk yarn was supplied to the market, thus the factories were not able to maintain their business viability. It is, therefore, necessary to carry out a study on model farm for Thai silk production under the community silk reeling factory system in order to support the production of commercial handicraft silk production, to increase the farmers' income as well as to supplement the imported silk yarn.

2. Research Objectives

2.1 To study the main components of a systematic Thai silk yarn production model for farmers' group under a community reeling factory.

2.2 To study and analyze the problems and obstacles for silk yarn production as well as to set up a mechanism that links the silkworm rearing and silk yarn production in order to obtain silk yarn qualities that meet the TACFS 8000-2005 standards.

2.3 To study the main factors which determine the success of cocoon production for the farmers under the networks.

2.4 To analyze the return on investment for Thai silk production under a community reeling factory system.

3. Scope of the Research

This study covered the community silk reeling factory for sericulture farmers group's network in Non-ngarm Village of Kudrang disitrict in Mahasarakam Province, the community silk reeling factory for sericulture farmers group's network in Nonghainoi Village of Nonsila District in Khon Kaen Province, and the community silk reeling factory for sericulture farmers' network in Haisok – Nongjikpattana Village of Puthaisong District in Buriram Province. This included the 3 community silk reeling factories mentioned above.

4. Expected Outputs

2.1 A community silk reeling factory model in order to scale up and develop the existing farmer group's networks to produce sufficient handicraft silk product to meet the need of the Thai silk industry systems.

2.2 A model system linkage among the silk production clusters, which should contribute to strengthening the small farmers and their groups to more sustainable development.

2.3 A system that links silk enterprise at a local level.

2.4 Increased silk quality standards of the farmers' groups under the factory networks to meet those of the TACSF 8000 – 2005.

2.5 Created job opportunities and income for farmers, and to support the economic and social security at a community level.

5. Review of Literature

Department of Agricultural Extension (DoAE) reported the general situation on Thai sericulture in 2004 that there were approximately 136,884 sericulture households, with the total mulberry planting area of 155,510 rai (or 24,882 hectares), (DoAE, 2004). The Department has 3 sericulture promotion models; Thai silkworm varieties, Thai hybrid, and Bivoltine (foreign varieties) (DoAE, 2002). DoAE's study on farmers' training under the sericulture extension project revealed that 90 percent of farmers applied their knowledge and technology to plan their sericulture farm practices while only 4 – 6 percent did not use the technology they learnt during the training (Direk, 2004).

The evaluation report of the sericulture extension project in 2002 for farmers in 10 provinces in different regions (the Northeast, North, West, South and the East) showed that of those farmers who attended the training on 10 sericulture techniques, 86.2 percent responded to the most common problem; i.e. silkworm diseases and insect pests (DoAE, 2003).

In addition, the report from the Devsilk Project emphasized the establishment of community silk yarn reeling factory to produce higher silk yarn quality than that of hand-reeled. The concerned sericultural extension centre was to produce and distribute good quality silkworm eggs to farmers under the network. The farmers reared silkworms and regularly fed cocoons into the factories. This was regarded as a comprehensive community sericultural practice (Devsilk, 2000).

A study was conducted at a community silk yarn reeling factory in Ban Non-ngarm of Kudrang District in Mahasarakam Province through 150 factory network members. The results of the study showed that 78.7 percent of the members held an average share of 2.48 in the factory, while 96 percent

responded that they were informed about cocoon price before each batch of silkworm rearing cycle. Eighty (80) percent of the respondents sorted their cocoon products before selling to the factory, and 88 percent received cash upon delivering cocoons for each cycle, with an average income of 6,372 Baht per year. Sixty (60) percent responded that they attended the network meetings 3 times per year. Among those respondents, 97.3 accepted that the community silk yarn reeling factory has contributed to an improved quality of life of the farmers (Suwit, 2003).

6. Research Methodology

6.1 Population

This study focuses two groups of populations;

- 100 farmers under the DoAE's Sericultural Extension Project, and belong to one of the three community silk yarn reeling factories in Buriram, Mahasarakam and Khon Kaen.
- Three farmers' groups under a community silk yarn reeling factory in Buriram, Mahasarakam and Khon Kaen.

6.2 Farmers Group Sample

The sample group was selected through a purposive selection process, i.e. to select sub-districts and districts where a community silk yarn reeling factory was located, and the sericulture farmers groups were members of the factory network in Buriram, Mahasarakam and Khon Kaen.

6.3 Data Collection

1. **Primary Data**; the primary data was collected through questionnaires from the chairs of 3 community silk yarn reeling factories, and 100 sericulture farmers under the factory networks.
   1) Farmers who were members of one of the three community silk yarn reeling factories in 3 provinces were selected;
      - Community silk yarn reeling factory in Non-ngarm Village of Kudrang District, Mahasarakam Province.
      - Community silk yarn reeling factory in Nonghainoi Village of Nonsila District, Khon Kaen Province.
      - Community silk yarn reeling factory in Haisok-nonjikpattana Village of Puthaisong District, Buriram Province. Number of selected farmers was 30 per province (Mahasarakam and Buriram) and 40 per province (Khon Kaen). Total number of farmers was 100.
   2) Meetings were organized for selected farmers to understand the contents of this study, and the required data based on data collection forms.
   3) Silkworm rearing plans were developed together with the farmers based on their capacity. Three rearing cycles were set;
      - Cycle 1: May 2007
      - Cycle 2: July 2007
      - Cycle 3: September 2007

4) Silkworm variety to be used was based on a common need among farmers group network under each community silk yarn reeling factory;
   - Community silk yarn reeling factory in Non-ngarm; an improved Thai silkworm variety (Dokbua)
   - Community silk yarn reeling factory in Nonghainoi; an improved Thai silkworm variety (Gold silkworm)
   - Community silk yarn reeling factory in Haisok-nonjikpattana; an improved Thai silkworm variety (Gold silkworm)
5) Silk production data was collected from both the farmer group members and the factory networks.
6) Silk yarn samples were drawn out from reeling process at the factories for quality testing.

2. **Secondary Data**; the secondary data was derived from related documentations, researches and related laboratory tests.

6.4 Data Analysis

Data derived from farmers and laboratories were analyzed and referenced with related researches.

6.5 Results

Results and Discussion

Results

1. Each of the 100 selected members of community silk yarn reeling factories reared at least one egg-sheet per cycle for 3 cycles. An average total cocoon yield derived was 51.04 kilograms, which was converted to an average income of 5,216.32 Baht per farmer (Table 1-3). Below is the result of each group;
   1.1 Forty (40) farmers from Nonghainoi community silk yarn reeling factory in Nonsila District of Khon Kaen province reared a Thai hybrid silkworm variety for 3 cycles, 1 egg-sheet per cycle per farmer. The average cocoon yield was 14.04 kilogram per egg-sheet, with the average production cost of 99.45 Baht per kilogram. The average price of cocoons was 100.06 Baht per kilogram, and the profit made was 0.61 Baht per kilogram. Each farmer obtained an average income from selling cocoons at 1,405 Baht per egg-sheet.
   1.2 Thirty (30) farmers from Haisok-nongjikpattana community silk yarn reeling factory in Puthaisong District of Buriram province reared a Thai hybrid silkworm variety for 3 cycles, 1 egg-sheet per cycle per farmer. The average cocoon yield was 17.37 kilogram per egg-sheet, with the average production cost of 117.15 Baht per kilogram. The average price of cocoons was 106.08 Baht per kilogram. The farmers made a loss of 11.07 Baht per kilogram. Each farmer obtained an average income from selling cocoons at 1,842 Baht per egg-sheet.

1.3 Thirty (30) farmers from Non-ngam community silk yarn reeling factory in Kudrang District of Mahasarakam province reared a Thai hybrid variety for 3 cycles, 1 egg-sheet per cycle per farmer. The average cocoon yield was 19.63 kilogram per egg-sheet, with the average production cost of 100.14 Baht per kilogram. The average price of cocoons was 99.91 Baht per kilogram. The farmers made a loss of 0.23 Baht per kilogram. Each farmer obtained an average income from selling cocoons at 1,969.32 Baht per egg-sheet

2. Each community silk yarn reeling factory has different capacity in producing silk yarn, depending two main factors; the quality of cocoons purchased from members, and skills and capability of the factory. The performance of each factory is outlined below;
   2.1 Nonghainoi community silk yarn reeling factory in Nonsila District of Khon Kaen province purchased cocoons of a Thai hybrid silkworm variety (Dokbua) from 40 farmers at 100.06 Baht per kilogram. The factory was able to produce a (cocoons : silk yarn) ratio of 11.53 : 1. The average profit made from this operation was 29.31 Baht per kilogram (Table 5).
   2.2 Haisok-nongjikpattana community silk yarn reeling factory in Puthaisong District of Buriram province purchased cocoons of a Thai hybrid silkworm variety (Golden silk variety) from 30 farmers at 106.08 Baht per kilogram. The factory was able to produce a (cocoons : silk yarn) ratio of 9.8 : 1. The average profit made from this operation was 152.33 Baht per kilogram (Table 6).
   2.3 Non-ngarm community silk yarn reeling factory in Kudrang District of Mahasarakam province purchased cocoons of a Thai hybrid silkworm variety (Golden silk variety) from 30 farmers at 99.28 Baht per kilogram. The factory was able to produce a (cocoons : silk yarn) ratio of 9.57 : 1. The average profit made from this operation was 311.06 Baht per kilogram (Table 7).

3. Silk Yarn Characteristics Produced;
   Silk yarn produced from the community silk yarn reeling factory, using manual-controlled small reeling machines of not more than 5.0 hp; the results of quality testing for elongation (%) tenacity (gram) and silk yarn size are as follows;
      3.1 Nonghainoi community silk yarn reeling factory in Nonsila District of Khon Kaen province produced silk yarn which has an average elongation and tenacity of 22.79 percent and 4.0 gram respectively, while an average size of the silk yarn was 234 Denier (Table 9).
      3.2 Haisok-nonjjkpattana community silk yarn reeling factory in Puthaisong District of Buriram province produced silk yarn which has an average elongation and tenacity of 23.32 percent and 4.9 gram respectively, while an average size of the silk yarn was 148.9 Denier (Table 10).
      3.3 Non-ngarm community silk yarn reeling factory in Kudrang District of Mahasarakam province produced silk yarn which has an average elongation and tenacity of 24.47 percent and

4.9 gram respectively, while the average size of the silk yarn was 156 Denier (Table 11).

Discussion

1. It could be generally observed that farmers obtained low percentage of silkworm survival rate. There were vast variations among cocoon production data that farmers obtained. Some farmers produced as low as 8 – 10 kilogram cocoons per egg-sheet. This low yield was believed to have caused by different factors, such as silkworm diseases (DoAE, 2003), transportation of egg-sheet, or environmental conditions. Intensive analysis on the root causes may be needed in order to improve the efficiency of silkworm rearing in each area so that the yield could be improved. Ideally, an average cocoon yield should not be lower than 20 kilogram per egg-sheet, so that the farmer could get a break-even on their investment.
2. Data on roduction cost and income (Table 4 – 7) clearly showed high cocoon production cost due to low yield obtained. This directly led to high production cost for silk yarn, especially where the cocoons as raw materials take not less than 80 percent of the total cost. Another interesting and important point to note was that of the income from by-products of the reeling process, i.e. fresh pupa. Thus, the results of this research led to a preliminary analysis that it is important to plan the silk yarn production process well that covers all aspects and concerns with all sectors to get the most.
3. The quality of silk yarn obtained from community silk yarn reeling factories met the universal silk quality standards; elongation (%), tenacity (gram). This was an important source of data for silk yarn production system in the future.

Conclusion and Recommendations

Conclusion

1. The average cocoon yield among farmers in three areas was 17.01 kilograms per egg-sheet per cycle. The lowest yield obtained was 8 kilograms per egg-sheet while the highest was 35 kilograms. The main cause of low yielding was silkworm disease attack during the 4^{th} and 5^{th} stage, which was in accordance with the findings in the evaluation of the sericulture promotion project in 2002 (DoAE, 2003).
2. The average silk yarn produced from the community silk yarn reeling factories was 54.64 kilograms per batch. This is a minimum quantity for a weaving enterprise to order on a commercial scale provided that the silk yarn quality meets the TACFS 8000 – 2005 standards (Seminar Proceedings; Sericulture Promotion Plans / Projects, and Annual Plan 2008; December 11 – 13, 2007, Amari Airport Hotel, Donmuang, Bangkok).
3. The promotion of networks for handicraft silk yarn production creates cash income and cash current within the community from the sale and purchase of fresh cocoons and silk yarn. This also creates job opportunity and a model for farmers' group development in the form of network systems; silkworm rearing groups, silk yarn reeling groups,

and silk weaving groups. The farmer groups systems can be developed along this line considering the main factors in the areas of new production technology management, group management (production, financial and marketing planning) as well as the network management for an effective operation.

4. The quality of silk yarn produced from the community silk yarn reeling factories, after testing at the Textile Analysis Centre, the Textile Industry Development Institute, was found to meet the international standards for its elongation and tenacity. The standard elongation should not be lower than 18 percent and the tenacity not lower than 3.7 grams. Thus, the silk yarn reeling systems that ensure the standard quality of silk yarn can be used as a source of raw materials for the silk weaving industry. This technical data can be used to support and guarantee the silk production at the community silk yarn reeling factory system (Table 8).
5. Sericulture farmer groups are able to obtain regular income throughout the year, and this could be treated as their monthly income. The cash flows within the community throughout the year, contributing to the community economic and building up a strong national economy.
6. The results of data analysis have shown that the models farmer groups under the community silk yarn reeling factory networks can produce handicraft silk yarn systematically due to the following factors;
   - 6.1 Each sericulture farmers' group consisted of not less than 30 members who are able to rear at least one egg-sheet per cycle and not less than 3 cycles per year (Table 4).
   - 6.2 The community silk yarn reeling factory should have not less than 480 kilograms of good quality cocoons in order to produce not less than 50 kilograms of good quality silk yarn per batch, in order to create a linkage with the weaving enterprise network on a commercial scale.
   - 6.3 The community silk yarn reeling factory should set up a system to produce good quality silk yarn, in order to place a quality control system for cocoons and silk yarn and to improve the product quality.

Recommendations

1. The linkage between the factory and the farmers' groups should be more systematic, and that the farmers are registered as members. That will lead to a strong network and membership for better production and development planning.
2. The concerned agencies should set up plans / programs to support the farmers' groups to produce the products that meet the TACFS 8000-2005 quality standards. This will clearly link to the outcomes in the form of farmers' income, and a handicraft silk yarn marketing system which feeds good quality silk yarn to the weaving industry.
3. It is necessary to provide support to the silkworm rearing groups under the community silk yarn reeling factory to increase their efficiency in producing good quality cocoons. Both cocoon producers and the reeling factories have to work compatibly in order to sustain their operations. A weak point at any end could lead to a problem, especially

4. Production plans should be drawn among the network members, the silkworm rearing groups and the reeling groups. Data from this study showed that there is a potential for the groups under the three reeling factory networks to increase their production capacity to more than one egg-sheet per cycle per farmer, or they could increase the number of cycles from 3 to 4. Under this study, each of the 30 – 40 farmers reared one egg-sheet per cycle to produce 54 kilograms of silk yarn. It is foreseen that the increase of production capacity means the silk yarn product could double to 60 – 80 kilograms.
5. The silk yarn reeling factories should improve their reeling techniques. This should vary depending on the types of cocoons purchased, which should match with the available machines. Cocoons should be sorted before reeling. The group should have a business plan to manage a small business.

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Development of Natural Dye Standard for Eri Silk.

Somying Chuprayoon[1] Sivilai Sirimungkarat[2] Dusit Pojun[3].
[1] Queen Sirikit National Sericulture Institute, Office of Permanent Secretary, Ministry of Agriculture and Cooperatives.
[1] Entomology Division, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University.
[1] Industrial Promotion Centre Region 5, Khon Kaen

Abstract

The objective this research on the development process of the natural dye standards for Eri silk was to establish a set of basic standards of dye stuff for Eri silk. The testing process was carried out with 16 dye-giving plants; *Tectona grandis* L.f., *Cassia siamea* Britt., *Terminalia catappa* L., *Diospyros mollis* Griff. , *Morus alba* Linn., *Erythrina variegata* L.,*Tagetes erecta* Linn., *Clitoria ternatea* Linn., *Peltophorum dasyrachis* (Miq.)Kurz., *Pterocarpus indicus* Willd., Artocarpus heterophyllus Lamk., Azadirachta indica A.Juss., *Garcinia vilersiana* Plerre., *Terminalia chebula* Retz., *Morinda coreia* Ham, *Thytocrene braeteata* Wall. and an insect; *laccifer lacca* Kerr. The dyeing process included both with and without mordant substances. The result of the above testing came 30 different colours on Eri silk yarn, 11 of which (3 samples without mordant and 8 samples with mordant) were further undergone durability test. The results were that 9 of 11 tested dyed samples showed light durability at levels 4 – 6 (good – excellent), and washing durability at levels 4 – 5 (good – excellent),10 of 11 samples showed their durability to fake sweat at both alkaline and acid states at levels 4 – 5 (good – excellent); 5 dyed samples showed their durability to wetness at levels 3 – 4 (medium – good). The results of the test indicated that it was possible to use natural dyes on Eri silk yarn and had durability standards at a good level provided that the right process was followed with at least 2 times dyeing. The use of mordant proved that the Eri silk yarn absorbed more dye stuff and gave brighter colour shades. A comparison testing on Eri silk yarn and fabric piece with red colour from *Laccifer lacca* Kerr. and yellow from *Garcinia vilersiana* Pierre. yielded no difference in terms of colour intensity. However, the piece dye on Thai silk yarn warp with Eri silk yarn weft showed higher colour intensity compared with that of both Eri silk yarn warp and weft.

Background

The use of natural dyes with cotton and natural silk fibre is a kind of local wisdom that has been practiced in Thailand for a long time. Most natural dye stuffs come from tree parts; barks, leaves, stems, roots, etc., as well as soil. These dyes give both bright and non-bright colour shades, and carry their valuable identity which is worth developed and studied. This is a valuable Thai wisdom which has adhered to the Thai textile. One good example is the indigo-dyed cotton cloth which is now well-known in Japan. For Thai silk yarn, farmers have used different types of dye stuffs, for example natural dyes, ready-mixed packaged chemicals, and chemical single-coloured dyes, or mixed (Somying, et.al.; 1988, 1992). Each type of dyes has different properties and durability on silk yarn. Thus, the result of each batch of silk dyeing may not yield exactly the same colour shade though the same group dye stuff is used. Natural dyes are extracted from different parts of different

kinds of plants which require specific steps and preparation processes. These are more complicated than those of chemicals, but if handling properly the brightness and durability qualities can be comparable. Natural dyes carry specific natural features and identity and are environmentally friendly. Therefore, the natural degumming and dyeing is suitable for textile product creativeness that show natural products which are safe and help to protect the environment.

Eri silk (*Philosamia ricini* B.) is a wild silkworm that yields a fur-like fibre, less lustrous compared to native silk yarn. The silk provides good sweat absorption and ventilation. Fabric made from Eri silk is light, soft, elastic and does not wrinkle easily (Sirimungkararat, et.al., 2002). The results from a primary research revealed that Eri cocoons are open-type which require a special reeling process. The cocoons are boiled in 3% Sodium Carbonate (Na_2Co_3) soluble water at 70 degree Celsius for 3 – 5 minutes. The boiled cocoons are stretched on a 25 cm x 25 cm square wooden frame, 10 – 15 cocoons each. After that, they are feathery before being spun to become silk yarn (Khobkoll, et.al., 2006). An alternative reeling process is through traditional hand-reeling equipment. Eri silk is suitable for the development of a safe natural commodity as from the start of silkworm rearing until the weaving process there is no use of harmful chemical. It is therefore deserved to be called ‘a green product’.
Though in Thailand Eri silkworm rearing has started and some products have been developed, there has been no development on natural dye standards in order to create product diversity as well as to add value to the commodity. Most of the basic knowledge on natural dyes centers mainly on dyeing of Thai silk yarn derived from mulberry silkworms. The Editorial Board (2001) presented 27 different kinds of plants which can be used for dyeing purposes. Kampon (2001) emphasised the steps in the dyeing and production of dark blue indigo-dyed fabric, called ‘Mor Nil Soaking’ in the Northeastern Thailand (Esarn), or ‘Mor Hom’ in the North. This is a kind of local wisdom worth conserving. Beside plants, natural dye stuffs can be derived from other natural sources. For example, lac insect (*Tachardia lacca* Kerr) is used for red colour. This is the only type of animal which can be used as natural dye stuff and has been practiced for a very long time. This knowledge has been inherited and become a local wisdom in Thai communities in the North and the Northeast (Kampol, 2001).
Dr. Motoi Minakawa, et.al., (1987) commented that the ability of fiboin fibre in wild silk to absorb dye stuff is lower than that of the mulberry silk. This is because the fiboin in wild silk contains amino acid with high alkalinity and that makes it difficult for Eri silk dyeing process. This fact is in line with M.S. Jolly, et.al., (1979) who confirmed that Eri silk had less lustrous feature compared with other types of silk and tended to shrink and wrinkle when washed, led to improper dyed. At present, the natural dyed Eri silk products have gained much interest and demand from the silk fabric consumers (Suchart Julpool, 2005). It is therefore necessary to carry out a research on development of natural dye standards for Eri silk to contribute to the benefits of the Thai textile industry.

Objectives

1. To gain effectiveness in natural dyeing of Eri silk yarn
2. To gain effectiveness in natural dyeing of Eri silk fabric
3. To develop the durability standard of natural dyes for Eri silk.

Research Sites

The research sites included the following;

- Nongyarplong village, Munjakiri District, Khon Kaen Province
- Industrial Promotion Centre Region 5, Khon Kaen.
- Department of Entomology, Faculty of Agriculture, Khon Kaen University.
- Roi-et Silk Products Groups Network
- Surin Silk Products Groups Network
- Textile Industrial Development Institute, Kluaynamthai, Bangkok
- Queen Sirikit National Sericulture Institute, Chatuchak, Bangkok

Methodology

1. Prepare 30 natural dye solutions from 17 types both plants and animals, each type required different preparation process. Each solution must go through filtration process to separate hard particles and to avoid any debris to contaminate the silk yarn (table 1-2)
2. Dye the Eri silk yarn with natural dye stuffs, totaling 30 colours from 17 types of plants and animals. There are three types of dyeing process;
   2.1. Single natural dye without mordant additive.
   2.2. Single natural dye with mordant additive.
   2.3. Multiple natural dyes, topping techniques with mordant additive.

Steps for Eri Silk Yarn Dyeing (yarn dye)

1) Degum the Eri silk to be used for dyeing process.
2) Soak the degummed Eri silk yarn into a natural dye solution which has been prepared.
3) Press the silk yarn with hands so that it absorbs the dye solution evenly.
4) Bring the pot containing silk yarn soaked with natural dye solution to boil at 90 – 95 degree Celsius for 25 – 30 minutes. During this time, turn the silk yarn all frequently to prevent it from breaking and unevenly dyed.
5) Observe if the silk yarn absorbs dye stuff fully, then wash it with clean water, press to dry and let it dry in shade.

In case the mordant additive is required, add it after the dyeing is finished and continue boiling for 15 minutes. Then, wash the dyed silk yarn with clean water, press and jerk to dry, let it dry in shade. The mordant is used to facilitate the Different types of mordant are used; white lime, red lime, alum and other natural substances such as the leaves of cowa tree, etc.

Steps for Eri Silk Fabric Dyeing (piece dye)

Two types of fabric are used for dyeing process; fabric made from Eri silk weft and Eri silk warp, and fabric made from Thai silk weft and Eri silk warp. The silk has undergone degumming process before weaving, then put through dyeing process for natural yellow (*Garcinia vilersiana* Pierre.), and natural red (*Laccifer lacca* Kerr.).

1) Wash the fabric for dyeing and dry.
2) Dye the fabric by soaking into a natural dye solution.
3) Press the fabric in the solution to help dye absorption.
4) From observation, once the fabric has absorbed the dye solution fully, bring the pot containing silk fabric soaked with natural dye solution to boil at 90 – 95 degree Celsius for 30 – 40 minutes, or until the absorption saturates. Bring the dyed fabric from the pot, add a mordant before returning the dyed fabric. Boil for another 10 – 15 minutes. Then let it cool down.
5) Wash the dyed fabric with clean water, press to release water and let it dry in shade.

3. Durability Test

Bring 11 samples (out of 30 colour samples) of dyed Eri silk yarn to test for 4 durability characteristics

3.1 Durability to light; to test against IS0 105-B02: 1994 (E)
3.2 Durability to washing; to test against IS0 105-C01: 1994 (E)*
3.3 Durability to artificial sweat; to test against IS0 105-E04: 1994 (E)*
3.4 Durability to brushing; to test against IS0 105-X12: 2001 (E)*

4. Colour fading primary test in Eri silk yarn at farm group level

4.1 Prepare silk fabric using degummed Thai silk yarn as warp
4.2 Select 5 colours (out of 11) of the dyed Eri silk yarn which has undergone the durability test to be used as weft. Between each colour, use natural degummed yarn to weave as intervals, 4-5 centimetres each, or as appropriate. The final product should be stripe silk fabric with 5 different colours with natural white in between.

Stripe 1 Green weft silk (*Garcinia vilers*+ *Clitoria tematea* Linn.+ white lime solution)
Stripe 2 Yellow weft silk (*Garcinia vilersiana* Pierre.+ alum)
Stripe 3 Red weft silk (*Laccifer lacca* Kerr., dyed twice) + Cowa tree + and the leaves of *Memecyon edule* Roxb
Stripe 4 Brown weft silk (*Thytocrene braeteata* Wall.)
Stripe 5 Orange weft silk (*Morinda coreia* Ham.)
Stripe 6 White weft silk (non-dyed)

4.3 Wash the striped fabric in a washing machine, spin dry, let it dry in shade and iron.

Results

1. Eri silk yarn dyeing with natural dye stuffs extracted from 16 types of plants and 1 type of animal yielded 30 colour dyed silk yarn; 27 single colours and 3 mixed colours. All 30 colours could be used with or without mordant. A one-off dye solution dipping resulted in low colour intensity and less evenness. This was in line with Dr. Motoi et.al., (1987) and M.S. Jolly, et.al, (1979) who reported that the dyeing process for wild silk and Eri silk

yarn was more difficult than yarn of mulberry silkworms. Thus, the dyeing of Eri silk should be done two times for each colour in order to obtain higher intensity and evenness(Table2and Picture1)

2. Eri fabric (piece) dyeing with natural dye stuffs
   2.1 Silk fabric made from Eri silk yarn weft and warp when processed as piece dye showed no difference compared with that of yarn dye. If higher colour intensity is required, the fabric should be dyed twice in the same dye solution. The use of a right combination of mordant and dye type helped stimulating colour intensity (Picture 2).
   2.2 Silk fabric made from Eri silk yarn weft and warp when processed as piece dye showed uneven colour shade and dye absorption tended to be more difficult. Colour intensity was also less than that of silk fabric made from Thai silk warp and Eri silk weft (Picture 3).
3. Durability quality standards for 11 natural dye stuffs on Eri silk yarn tested at the Textile Testing and Analysis Centre, Textile Industry Development Institute showed that one of the total did not pass the durability to light quality standards, i.e. Level 2 (relatively low). Two of those did not pass durability to brushing quality standard – colour fading to wet white cloth, i.e. Level 2 (relatively low), and Level 1 (low), (Table 3)

The data from the results of Eri silk dye durability qualitystandards tested at the Textile Testing and Analysis Centre, applying the ISO/ IEC17025 – 2005 standards from the Office of Industrial Products Standards, enabled the natural dye standards for Eri silk to be primarily set up. This included; quality standards for dye durability to light, quality standards for dye durability to washing, quality standards for dye durability to artificial sweat, and quality standards for dye durability to brushing. These were at Level 4-5 (good to very good).

The Eri silk natural dyeing process must be done properly for at lest 2 times. The use of dye solution extracted from leaves, flowers and fruits should be mixed with mordant as an additive to stimulate the dye absorption. Though the solutions from barks, stems and roots gain much absorption, the use of mordant would help better off. Moreover, the ratio between raw materials from plants and animal to water, and that of dye solution to silk yarn weight becomes a co-factor that determines the dye durability quality standards

4. A simple quality standards testing for dye durability (colour fade) of Eri silk products at farm level could be applied by piece dye technique. The farmers may weave silk fabric in stripe pattern using different colours, weaving a white stripe between each colour. The quality standards for dye durability to washing test is done by washing this coloured stripe fabric in a washing machine. Inspect if any colur fades to any white stripe woven interval, (Picture 4).

Discussion

1. Eri silk yarn produced nowadays comes in a wide range of quality and sizes as well as the evenness of the thread. As a result, this delays the development of dye standards because many repetitions are required in order to confirm the results of the research on the effectiveness of dye absorption. In addition, the quality of Eri silk yarn also determines the type of weaving equipment to use. For example, the use of weaving comb that

has an appropriate spacing so that it does not create a weaving problem. If weaving comb spacing is not in align with the size of silk yarn, it is most likely that the Eri silk warp will break or become feathery after weaving. If the Eri silk is used as weft, this will result in getting unevenly stripe fabric which is also an obstacle to the research works.

2. Some types of natural raw materials are not always available all year round. Some only bear fruits once a year, e.g. *Diospyros mollis* Griff.etc. This has a direct effect to the dealy of this research and poses a direct impact onto the development of natural dye standards for Eri silk.
3. There were uncontrollable factors during this research works. The stability of plant and animal parts was one of the main factors. Most of the plants grow naturally at different ages, while lac insect raised in different locations did not give the same quality. When prepared as dye solutions, this quality has a direct effect to dye absorption and colour intensity. As a result, this research work took longer time for the preparation of raw materials so that there was more control over this factor to make the research more complete.

Research Summary

1. Eri silk yarn is a product derived from non-mulberry silkworms which can be dyed with natural dye stuffs. Dye absorption in Eri silk yarn is more difficult than that in regular Thai silk yarn derived from mulberry silkworms. Therefore, it is advised that dyeing is done twice to obtain more colour evenness and intensity. In addition, different parts of plants give different quality of dye solutions. Flowers and leaves especially are not suitable to be used for single colours because their durability and colour absorption will be at Level 2 (relatively low). Thus, they should be mixed with dye solutions from other plant parts, e.g. dye from butterfly pea flowers should be used together with that from leech lime tree, etc. The use of mordant may be necessary in some cases. For example, dye solution from *Myrobalan* leaves should be added with copper sulphate(blue vitrol) as a mordant to increase the dyeing effectiveness. It could be said that to obtain natural dye standards for Eri silk dyeing one must consider using the right dye stuffs together with the right combination of mordant as an additive substance.
2. In comparison with Eri yarn dye, the natural Eri silk piece dye on fabric made from Eri weft and warp is relatively more difficult, while there is no difference in colour intensity. Dye absorption and evenness in piece dye is better than that of yarn dye as the silk thread is uniformly put in order of the fabric structure. Silk yarn in skein form has no uniformity among the threads and dye absorption effectiveness depends on yarn and dye contacts.
3. The most suitable technique for Eri silk natural dye is 'hot dye' so that the dye is absorbed in to the silk yarn more effectively. This technique starts from cold dye to prevent colour unevenness. Later, increase the temperature until it reaches 90 – 95 degrees Celsius. One precaution is that the silk must be over-turned regularly while the dyeing is in progress. This is to prevent the silk yarn to become feathery and messy. The use of mordant must undergo boiling as well (Table 3).

4. The quality standards for natural dye durability in Eri silk are in Level 4 – 5, i.e. Good – Very Good under the ISO 105-B02: 1994 (E), ISO 105-C01: 1994 (E)*, ISO 105-E04: 1994 (E)* and ISO 105-X12: 2001 (E)*. Therefore, the natural dyed Eri silk products can to be used to produce garments or other ready-to-use products, as well as household textile, e.g. furniture upholstery, etc. Natural dyes from Tongarn and Smore are the only ones that are not suitable for household textile as there quality standards for durability to brushing is at Level 2 and 1 respectively (Table 3).
5. From the results of this research on the development of natural dye standards, two types of Eri silk products can be made available; natural Eri silk product, and natural dyed Eri silk products.
   5.1 Natural Eri silk products are those undergone through all processes using natural substances from degumming, dyeing and the use of mordant.
   5.2 Natural dyed Eri silk products are those undergone through natural dyeing process, but may use chemicals during degumming and mordant additive.

   The two types of products are called natural Eri silk products with quality standards for their durability (Table 3).

Recommendations

1. The Eri silk yarn quality should be developed and the technology on Eri silk yarn production (direct yarn reeling and cocoon spinning techniques) to be transferred in order to gain holistic standard development for Eri silk.
2. Support to farmer groups network who produce Eri silk should be made holistic; i.e. for farmers who rear, reel, process Eri silk. This can be done in as a pilot project to develop and set up an appropriate system to enable a farmer groups networks to function.
3. Support should be made available for researches on natural Eri silk development in order to gain technical data to facilitate the development of Eri silk production plans to concretely upscale the production at farmer groups and other interested persons.
4. There should be policies, implementation plans and projects to support the development of Eri silk products continuously, as well as to add value to the Eri silk products and to create income for communities.
5. PR plans and dissemination of information should be made systematically and concretely so that market expansion for the products is more sustainable and secure. Meanwhile, it can create more confidence among cassava growers or other interested individuals who want to pursue Eri silk products.

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Table 1 List of Plants and Animals used in Dye Solutions Preparations

Common Name	Scientific Name	Plant Part
Jackfruit	*Artocarpus heterophyllus* Lam.	hardwood
Cassod tree (Thai copper pod)	*Cassia siamea* Britt.	leaves
Tonghan	*Thytocrene bracteata* Wall.	stems
Marigold	*Tagetes erecta* Linn.	flowers
Indian coral tree	*Erythrina variegate* Linn.	flowers
Burma Padauk	*Pterocarpus indicus* Willd.	bark
Leech lime	*Garcinia vilersiana* Pierre.	bark
Ebony tree	*Diospyros mollis* Griff.	fruit
Burmese rosewood	*Morinda coreia* Ham.	root
Myrobalan	*Terminalia chebula* Retz.	leaves
Neem tree	*Azadirachta indica* A. Juss.	leaves
Teak wood	*Tectona grandis* Linn.f.	leaves
Mulberry	*Morus alba* Linn.	fruits
Indian almond tree	*Terminalia catappa* Linn.	leaves
Copper pod	*Peltophorum dasyrachis* (Miq.) Kurz.	bark
Butterfly pea	*Clitoria ternatea* Linn.	flowers
Lac insect	*Laccifer lacca* Kerr.	stick lac

Table 2 List 30 natural colour dyed on Eri Silk Yarn/

No	Colour	Plant / animal used	Part	Mordant
1.	Yellow	Jackfruit	Hardwood	Alum
2.	Light yellow	Jackfruit	Hardwood	-
3.	Yellow	Cassod tree	Leaves	-
4.	Dark green	Cassod tree	Leaves	White lime solution
5.	Brown	Tonghan	Stem	White lime solution
6.	Yellow	Marigold	Flowers	-
7.	Light orange	Indian coral tree	Flowers	-
8.	Brown	Burma Paduak	Bark	-
9.	Grey	Burma Paduak	Bark	Mud
10.	Yellow	Leech lime	Bark	Alum
11.	Silver grey	Ebony tree	Fruits	-
12.	Yellowish orange	Burmese rosewood	Roots	-
13.	Light yellow	Myrobalan	Leaves	-
14.	Yellowish green	Myrobalan	Leaves	Copper sulfate
15.	Black	Myrobalan	Leaves	Alum + rusted iron
16.	Light brown	Neem tree	Leaves	-
17.	Dark brown	Neem tree	Leaves	White lime solution
18.	Light brown	Teak wood tree	Leaves	-
19.	Dark brown	Teak wood tree	Leaves	Red lime solution
20.	Purple grey	Mulberry	Berries	-
21.	Yellow	Indian almond tree	Leaves	Alum
22.	Brown	Copper pod	Bark	-
23.	Dark brown	Copper pod	Bark	White lime solution
24.	Greenish grey	Butterfly pea	Flowers	White lime (no boiling)
25.	Lead Grey	Butterfly pea	Flowers	White lime (boiled)
26.	Green	Leech lime+butterfly pea	Leaves+flowers	White lime (no boiling)
27.	Yellow	Leech lime+butterfly pea	Leaves+flowers	White lime (boiled)
28.	Light yellow	Leech lime+butterfly pea	Leaves+flowers	Red lime (boiled)
29.	Light red, pink	Lac	Fresh lac	Cowa leaves + *Memecyon edule* Roxb
30.	Dark red	Lac	Fresh lac	Cowa leaves + *Memecyon edule* Roxb

Table 3 Durability Quality Standard Test of Natural Dye for Eri Silk

Type of natural dye/ Mordant Durability test	Garcinia vilersiana Pierre+C.terrmatea L.+white lime (not boil)	Garcinia vilersiana Pierre+C.terrmatea L.+white lime (boil)	Garcinia vilersiana Pierre+C.terrmatea L.+red lime (boil)	Terminalia chebula Retz.	Morinda coreia Ham.	Garcinia vilersiana Pierre+ Alum	Laccifer lacca Kerr.+ Cowa leaves	Laccifer lacca Kerr.	Thytocrene bracteata Wall.+ White lime	Terminalia chebula Retz.+Alum+Rustiron	Terminalia chebula RETZ.+Copper sulphate	rer
1. Light - Level	2	4	4-5	2-3	4	4	5	5	5	5-6	4	- I 105 BO 94(
2.Washing: (Level) : colour change : colour fading to white - Cotton	4-5 4-5 4-5	4 4-5 4-5	4 4-5 4-5	3-4 4 4	4 4-5 4-5	4 4 4	4 4 4	4 4 3-4	4 4 4	4 4 4	3 4 4	- IS 105 CO 989

- Silk											- IS 10 EO 19 (E)
3. Sweat (Level)											
- acid	3	4	4	4	4	4	4	3-4	4	4-5	
: colour change	4	4-5	4-5	3-4	4	3-4	3-4	4	3-4	3-4	
: colour fading to white	4-5	4-5	4-5	3-4	3-4	3-4	3	3-4	3-4	3	
- Cotton	4	4	4	4	4	3-4	4	4	4	4-5	
- Silk											
- alkali	4	4-5	4-5	3-4	4	4	3	4	3-4	4	
: colour change	4-5	4-5	4-5	3-4	4	4	3	4	3-4	3-4	
: colour fading to white											
- Cotton											
- Silk											

4. Brushing(Level)	4	4	4	4	4	4	3-4	3-4	3	2-3	4	- IS 105
: colour fading to dry white cloth	3-4	3	3	2-3	3-4	2-3	3	2-3	2	1	3	X12 01
: colour fading to wet												

Note : *durability quality standards tested at the Textile Testing and Analysis Center, applying the ISO / IEC 17025-2005

Dyeing of Tropical Tasar Silk Textiles with Lac Dye

Z. M. S. Khan, N. S. Gahlot, N.G. Ojha and N. Suryanarayana*
Central Tasar Research & Training Institute, Central Silk Board
P.O. Piska-Nagri, Ranchi - 835 303, India

ABSTRACT

Lac is one of the most valuable gifts of nature to mankind and in earlier days lac dye was extensively used on animal fibres like wool and mulberry silk for dyeing. Introduction and commercialization of synthetic dyes during the middle of 19th century triggered the gradual decline in usage of natural dyes and were replaced by synthetic dyes. Once again environmental awareness throughout the world now seems to catalyse the usage of natural dyes in the industry. Health hazards due to usage of synthetic dyes and chemicals in Textiles have forced environmentalists to think in terms of reviving the usage of natural dyes in textile industry. Taking into consideration the above facts, the usage of lac dye for dyeing tasar silk yarn and fabrics was planned. Tropical tasar silk contains its own natural fawn colour which is having high popularity among the consumers of tasar silk. However, aiming towards diversified product and making tasar more attractive and diversified, tasar fabrics were dyed with lac dye using different mordants. Twelve different colours and shades have been developed on tasar fabrics. It is observed that the use of concentrated lac dye with mordant in dyeing of tasar textiles resulted in deeper shade with good wash fastness and twelve attractive natural shades with different color were obtained by using different mordants with lac dye. The use of lac dye for dyeing of tasar silk yarn and fabric is commercially viable and techno-economically feasible. These natural shades can be exploited in all tasar producing States in weaving, dyeing clusters and in different new fashion industries for product diversification.

KEY WORDS: *Lac dye, Mordant, Tasar yarn, Degumming, Bleaching, Fastness properties.*

INTRODUCTION

The Lac dye is obtained as a by-product of Shellac industry. It is extracted from Kerria *lacca insect.* The forest area nearby Ranchi (India) has a big potential of insect *Lacca*. Lac dye is a derivative of lac and has been in use for coloring food and fabrics since ancient times. Lac is a unique material of animal origin being the secretion of a tiny insect *Kerria lacca* (Kerr) thriving on certain host plants viz., Palash (*Butia monosperma*) Kusum (*Schleicherm oleosa*) Ber (*Zizyphus mauritiana*) etc. Lac dye is an important by product of lac industry and is easily recoverable and is produced in large quantities during lac manufacture (Ghosh, A.K.and Sengupta 1977, Prasad *et al.*1998). Different attractive colors and shades are obtained by using different mordants with lac dye (Gupta *et al.* 1991).It has been observed from earlier research that different shades of colouring could be achieved by using different moardants. However, detailed studies are required on application of lac dye on tasar silk fabrics. As Germany struck a severe blow the dyestuff industry in late 1994,by import of textile garments coloured with series of azo based dyes. It has been decided to re-exploit dyes of natural origin, which are non-toxic as well as eco-friendly. Now a day's textile

industry is in need of safer dyes and demand for diversified products to meet the buyer's needs is growing day by day. Hence, the present study is an attempt to understand and develop a suitable package of dyeing of tasar silk fabric using lac.

MATERIALS AND METHODS

Materials

A plain weave loom state tasar silk fabric (reeled x reeled yarn) having 60 ends/inc and 77 picks/inch with 55 gm/sq.m in fabric weight and Lac dye was collected from Indian Lac Research Institute, Namkum, Ranchi, India and used as experimental material without purification. Anhydrous Glauber's salt and Acetic acid of L.R. grade were used as the dye bath assistants. Lac dye was used different concentrations depending upon the intensity of colour and shade required. The permitted mordants namely Potash Alum, Tin chloride, Potassium dichromate, Cupric sulphate, Oxalic acid were used in different percentage depending upon intensity of shade required.

METHODS

Degumming:

Degumming of tasar silk yarns and fabrics was carried out by using permitted H_2O_2 concentration and neutral soap.

Recipe:-

1st Bath	2nd Bath
Soap -15%	Soap -15%
Soda -5%	Soda - 5%
Boiling time - 45 mins	Boiling time- 20-30 mins
M: L - 1:40	M: L - 1:40

Bleaching:

Bleaching of tasar silk yarns and fabrics was carried out by using permitted concentration of H_2O_2 (chlorine bleaching is banned e.g. Chlorophenols heavy metal compounds, halogenated solvents, fluorocarbon, quaternary ammonium compounds, ammonia treatment, oxygen bleaches, chlorination, optical brightening agents etc.).

Recipe:-

Hydrogen Peroxide - 12cc/ litre
Sod. Silicate - 2 gm/litre
Sod. Perborate - 1gm/litre
Liquor Ratio - 1:40
Temperature - 60°C
Time - 40- 60 mins.

Mordanting:
Degummed and bleached tasar fabric is first mordanted with the following recipe:
Material: Liquor – 1:40
Temperature – 80^0C to 60^0C
Duration – 30 minutes

Dyeing:
Mordanted tasar fabric is then dyed in the same bath with the following recipe:
Glauber salt : 10%
Formic acid : 3-4%
Temperature : Boiling
Time : 60 minutes

Under the study following combinations of mordants and lac dyes were used

Sl.No.	Sample code	Mordants	Lac Dye
1	LCM-1	Potash alum 2%	0.5%
2	LCM-2	Potash alum 2%	1.0%
3	LCM-3	Tin Chloride 2.0%	5.0%
4	LCM-4	Potassium dichromate 2% and Oxalic acid 0.5%	1.0%
5	LCM-5	Cupric sulphate 2%	5.0%
6	LCM-6	Pot. dichromate 2%	0.5%
7	LCM-7	Copper sulphate 0.5%	0.5%
8	LCM-8	Tin chloride 0.5% Potash alum 0.5%, Oxalic acid 0.5%	0.5%
9	LCM-9	Pot. Dichromate 5.0%	5.0%
10	LCM-10	Potash alum 0.5%	0.5%
11	LCM-11	Pot. Dichromate 5.0%	0.5%
12	LCM-12	Tin Chloride 0.5%	0.5%

After dyeing with the above combinations of mordants and dyes the dyed materials were washed in 2% soap solution, rewashed in plain water and dried in shade. All the dyed samples were then tested for colour fastness against light, rubbing, perspiration and washing.

RESULTS AND DISCUSSION
Mordants as such have colour fixing effect and hence the pretreatment had a reaction which occurs between the lac dye and tasar fabric. The fabric is impregnated with the mordant, then during the dyeing process the dye reacts with the mordant, forming a chemical bond and attaching it firmly to the fabric. Table- 1 represents the test results of lac dyed fabric samples. Washing fastness for the entire lac dyed samples were at the rangeof 3-5, Based on scoring index analysis, samples LCM-8 and LCM-12 have performed the best towards colour fastness tests, followed by LCM-11, LCM-7, LCM-6 and LCM-10 pertaining to colour fastnessstsagainst wash, light, rubbing and perspiration. The light fastness was at the range of 2-4 and the rubbing fastness was at the range of 3-5. Test results of 12 combinations obtained were quite acceptable wash fastness and encouraging to promote

popularization and diversification of lac dyed tasar products in different clusters of tasar producing Indian States viz., Jharkhand, Chhatisgarh, M.P., Orissa, Bihar, West–Bengal, Andhra Pradesh, Maharastra, Uttar Pradesh and Uttranchal.

Table-1: Fastness results of lac dyed fabric samples

Sample Code	Wash			Light	Rubbing		Perspiration					
							Acid			Alkali		
	CC	SC	SS	CC	Dry	Wet	CC	SC	SS	CC	SC	SS
LCM-1	3	4/5	4/5	2/3	4/5	4/5	´Y	´Y	4	3	´Y	2/3
LCM-2	3/4	4/5	4/5	2/3	4/5	4/5	3	´Y	3	3	´Y	´Y
LCM-3	3/4	4/5	4/5	3	4/5	4	3	2/3	2	2/3	2	2
LCM-4	4	4/5	4/5	3	4	3/4	´Y	´Y	3	´Y	3	3
LCM-5	3	4/5	4/5	3/4	4/5	4	3	3	2/3	3	3	2/3
LCM-6	3/4	4/5	4/5	2/3	4/5	4	4	4/5	4/5	´Y	4	4
LCM-7	2/3	4/5	4/5	3	4/5	4/5	´Y	4/5	4	´Y	4/5	4/5
LCM-8	**3**	**4/5**	**4/5**	**3**	**4/5**	**4/5**	**4**	**4/5**	**4/5**	**4**	**4/5**	**4/5**
LCM-9	3	4/5	4/5	3	4/5	4	3	3	3	3	3	3
LCM-10	3/4	4/5	4/5	2	4/5	4	´Y	4/5	4	3	4/5	3
LCM-11	3/4	4/5	4/5	3/4	4	3/4	4	4/5	4/5	4	4/5	4/5
LCM-12	**3**	**4/5**	**4/5**	**3**	**4/5**	**4/5**	**4**	**4/5**	**4/5**	**4**	**4/5**	**4/5**
Abbreviation: CC ＋ Change in colour, SC＋ Stain on cotton, SS＋ Stain on silk.												

CONCLUSION

The findings deduce clearly that twelve attractive shades with different hue color can be obtained by using different mordants with lac dye. Hence, the uses of lac dye for dyeing of tasar silk yarn and fabric is commercially viable and techno-economically feasible. These natural shades can be exploited in all tasar producing States in weaving, dyeing clusters and in different new fashion industries for diversified product.

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Product Innovations With Indian Non-Mulberry Silk and Technologies for Fashion Apparels

Prof.Dr.M.Sivakumar , Expert Post Cocoon Technology, Bangalore, INDIA

India produces five commercial varieties of Silk viz-Mulberry silk, Tropical Tasar silk, Temperate Tasar Silk (Oak silk), Muga silk and Eri silk. Eri silk is a staple fibre while other types are continuous filament. Report says that the use of Silk and silk based fabrics, garments, Home furnishings are rising globally. One of the ways of increasing value addition is through Diversified product development from the traditional Indian silk yarns.

Dr.Hiromu Akai, president of International Society for wild silk moths has reported in Indian Silk-Jan-Feb 2006, that the wild silk have special health related characteristics. Eri and Antherea pernyi have anti-bacterial functions. Kimonos are produced in Japan from tasar Silk and are patented. An exclusive unique product from wild silk will have demand especially in western market.

Mulberry, Tasar and Muga silks are available in Continuous filament yarn form and Spun yarn forms. Eri silk unlike any other varieties of silk is available as cut staple fibre as the Eri cocoons are open mouthed and not suitable for reeling in continuous form. There are two varieties of Eri cocoons- white cocoons and Brick red cocoon. Tropical tasar cocoons are available in different eco races with different colours which are natural and fast. Main varieties of tasar silk goods exported from India are dress materials, sarees, ready made garments, carpets followed by sarees. Tasar silk fabrics are mostly used as gents wear in our country. The other outlets are in the form of thicker varieties like dress material and furnishing fabrics which are made from Tasar ghicha, Tasar-ghicha-noil and tasar katia yarn mostly catering to the export markets.

Shirting

Tasar Reeled silk x Tasar Reeled silk, Tasar Reeled silk x Mulberry reeled silk,

Tasar Reeled.silk x Tasar Ghicha ,Tasar Reeled silkx Katia, Tasar Reeled X Balkal (from cocoon Peduncle),Tasar Reeledsilk X Cotton

Sarees:

In Indian sub-continent saree is a vary popular dress wear among ladies which are produced in the following combinations.

Tasar reeled x tasarReeled (With plain boarded),Tasar reeled x tasarReeled (With tie and dye disigne),Tasar reeled x tasarReeled (With stripes and checks),Tasar reeled x tasarReeled (With Prints),Tasar reeled x tasarReeled (Jacquard design).

Chadar/Wrapper

Chadar or Wrapper fabric for covering in all the seasons: Tasar Reeled x Tasar Reeled (With plain, twill & diamond weaves), Katia x

Katia (Plain & Twill), Tasar reeled x katia, Jhuri x Jhuri (Heavy weight)

Export varieties/Furnishings :
Reeled tasar x Noil or spun silk yarn, Reeled Tasar x Ghicha. Reeled tasar x Ghicha + Noil, Cotton x Ghicha & Noil, Spun Silk x Ghicha & Noil., Jhuri x Jhuri ,Cotton x Jhuri (Double cloth)

Spun Silk Yarn-120/2 and 60/2nm 140/2nm and 210/2nm undyed or dyed Silk Noil Yarns,Bleached Tussah Silk Noil Yarns

Spun silk-Modal yarn-,Spun silk-dyed fabric –is made from different coloured yarns, creating a variety of coloured patterns., Doupion fabric-Doupion fabric is a plain-weave fabric with slubbed ribs. It has a stiff, taffeta-like hand and is usually dyed in bright colors.

Noil -Silk noil is made from the short fibers left after combing and carding so it doesn't shine like many other silk fabrics. Noil looks similar to cotton, but has the soft feel of silk against the skin. It also drapes better than cotton and resists wrinkling.

Silk, with its shimmer and lustre -- no other fabric can match the qualities of this rich fabric
Contact e-mail ID : sivasathy@yahoo.com

F. ECONOMY SECTION

Product Diversification - an Alternative for Sericulture Development in the Black, Caspian Seas and Central Asia Region Countries.

P. I. Tzenov & E. A. Kipriotis, Black, Caspian Seas and Central Asia Silk Association (BACSA), 5 A. Stamboliiski Str. Vratza 3000 Bulgaria.
Web: www.bacsa-silk.org
e-mails: panomir@yahoo.com ; nagrefk@otenet.gr

ABSTRACT

The sericulture industry has been a common culture of the Eastern Europe and Central Asia (Black, Caspian seas and Central Asia, BACSA) region countries for thousands of years. The main common problems for all the region are too low raw silk quality produced presently, resulting in fail to receive the standard price in the markets and to loose existing markets and that the production technologies at the field level are still more relying on the traditional technology and management system thus not to be commercially oriented operation. As the main disadvantages for sericulture development in the BACSA region countries may be identified the impossibility to compete without state support with China and other developing countries with respect to the cocoon/raw silk productional costs and the impossibility to compete with Italy and France in silk fabrics and commodities printing and design. The main advantages are the availability of comparatively rich mulberry and silkworm genetic resources, well developed science and technology in sericulture, the possible scientific cooperation among the BACSA and other countries through the European framework programmes, the strong state support to sericulture in some of the region countries leading to high competitiveness of their cocoons/raw silk at the market, recently attracted foreign investors in the silk industry, the developing silk market in the region, still available unique silk handcrafts production. The main directions of the regional strategy for sericultural industries revival and development may be the development of new sericultural products such as use the mulberry and silkworm for non – silk purposes, establishment an European mulberry and silkworm germplasm preservation center in one of the member states through the EU financial support programmes, establishment the BACSA countries as sources of sericultural genetic resources for the developing countries, production in bi/multilateral cooperation mulberry sapling of highly productive varieties and high quality silkworm eggs, production of very high quality bivoltine silk with certain special characteristics, silk handcraft production for selling to the tourists at the local market and also for export.

Keywords: **sericulture, silk, Eastern Europe, Central Asia, BACSA, strategy, diversification, development.**

1. Introduction

The sericulture industry has been a common culture of the Eastern Europe and Central Asia region countries for thousands of years. The preconditions/prospects for sericulture revival and development in the Black, Caspian seas and Central Asia (BACSA) region are such as very long tradition and experience in sericulture, availability of rich mulberry tree

resources, favorable climatic conditions, governmental support to the sector, the increasing demand within the European countries for natural and biological products along with the close huge European silk market etc.

Until the near past the BACSA region had an annual fresh cocoon production around 50,000 tons and occupied the third place in the world after China and India and nearly one million farmer's households were engaged with the sericulture. The BACSA member countries are still one of the poorest in Europe and with comparatively high unemployment therefore the sericulture industry development is amongst the highest priorities of the local Governments and European Commission as a way for contribution to poverty alleviation and food security through a quick increase of the incomes without big capital investments especially in rural and semi-urban area. The Central Asian region, including Iran, Tajikistan and Uzbekistan has been and still is the highest cocoon and silk producer, engaging in this industry more than 450,000 farmer's households.

The recent constraints, facing the sericulture industry development in the region may be divided as common for all the countries and specific for the European and Central Asian countries. The main common problems for all the region are too low raw silk quality produced presently, resulting in fail to receive the standard pricein the markets and to loose existing markets and that the production technologies at the field level are still more relying on the traditional technology and management system thus not to be commercially oriented operation.

On the other hand as a main barrier in the East European countries for their sericulture development is that presently the too low raw silk prices at the international market can not stimulate the farmers to produce cocoons without additional support from the government.

By the same time in most of the Central Asian countries the quality of presently available silkworm eggs do not meet the international standard and their quantity can not satisfy the local needs. For example in the recent years Tajikistan imports about 90 % and Uzbekistan - 60 % of the necessary silkworm eggs.

One of the basic aims of expansion of the inter – regional cooperation is therefore to transmit the richer sericulture germplasms and advanced technologies from the West to the East and simultaneously the East European countries to play a role as a bridge for opening the European silk market to the silk products both from Eastern Europe and Central Asia.

2. Reasons for the decline of cocoon and silk production in the BACSA region during the period 1990 - 2000.

The main reasons for cocoon/raw silk production decline were as follows:

- The transitional period from centralized to market economic system which lead to sudden stop of the governmental support to sericulture, breakage of the traditional economic relationships between the countries thus the destroy of the already established system for sharing the different parts of sericultural production.
- The raw silk price at the international market has been dictated by China and only few other low income countries could withstand the competition without state subsidies for the cocoon producers.

- The appearance of new, competitive with the silk synthetic fibres and their huge distribution in the international market.
- The instability of cocoon and silk prices particularly within the local markets.
- The rapid urbanization due to heavy industrialization, especially in Eastern Europe.
- The land re-distribution policy and crop re-structuring especially in areas where sericulture was popular and active and mulberry plantation had a big share among crops.
- The gain of new crops, especially industrial ones, which provided higher incomes and lead to the replacement of the mulberry plantations.
- Loss the traditional silk markets in Russia and Baltic countries and difficulties in exploring new markets. In the silk carpet producing countries-the difficulties on exportation of silk carpet and also smuggling very cheap silk yarn.
- Comparatively low raw silk quality produced, due to lower cocoon quality and out of the date old silk reeling machines and technology.

- It is not possible to provide training opportunities for handicrafts and this causes a quality loss in workforce. To create new designs, patterns and conception suitable for nowadays is failed.
- Selling cheap and low quality carpets especially coming from Far East with prevalence of traditional Persian designs could not be prevented. As the genius hand woven silk carpets didn't have a chance to compete with the others owing to their high prices, many companies left the market, expanded usage of machine-made or other synthetic rugs.
- Reduction and/or limitation of international economic growth rate which encourages utilization of cheaper products.

3. The silk commodities production and trade in the BACSA region countries

3.1. The silk commodities, produced in the BACSA region.

The silk commodities, produced may be divided roughly in two groups, namely industrially produced silk commodities and silk handcrafts.

The industrially produced items are silk and blended with other fibers fabrics and garment. The main producers are Greece, Uzbekistan, Azerbaijan, Bulgaria and Romania.

Silk carpets and rugs are the most famous silk handcrafts in the BACSA region whose producers are Iran, Turkey, Afghanistan, Uzbekistan, Turkmenistan, Tajikistan, Kyrgyzstan and Azerbaijan.

Handloom woven silk fabrics, embroidery, knitted garment, souvenirs etc. are produced in small quantities in nearly all the region countries.

3.2. The silk commodities trade in BACSA region

As regards the silk trade, the BACSA region countries could be divided into 3 groups, namely: **1) countries mainly silk exporters; 2) countries mainly silk importers** and **3) countries which do not produce significant silk amount, but do not import any significant silk quantities as well.**

As main silk exporters could be identified Uzbekistan, Iran and Turkey. The total approximate value of the silk export of the region in 2004 is about 600 million US$, 97 % out of this sum is accounted to the silk carpets export by Iran and Turkey. The main silk importer is Greece.

4. The disadvantages and advantages of the BACSA region countries for their sericulture revival and development.

As the main disadvantages for sericulture development may be identified:

- The impossibility to compete without state support with China and other developing countries with respect to the cocoon/raw silk productional costs.
- The impossibility to compete with Italy and France in silk fabrics and commodities printing and design. Presently no any manufacturer can compete with the Italian silk printers for ready printed goods. The Italians are much more flexible to changes in fashion.

The main advantages are:

- Availability of comparatively rich mulberry and silkworm genetic resources, well developed science and technology in sericulture. In 2006 and 2007 BACSA organized in 4 countries simultaneously an international testing of 15 F_1 silkworm hybrids, originated from 10 different countries which proved that after the Japanese hybrid some of the local hybrids from Bulgaria, Ukraine, Turkey, Azerbaijan, Romania and Uzbekistan manifested the highest silk productivity.

- The possible scientific cooperation among the BACSA and other countries through the European framework programmes.

- The strong state support to sericulture in some of the region countries: Recently most of the region countries gradually solved the economical problems, caused by the transition from a centralized to a free market system, thus the governments increased their support to the silk industry. The silkworm rearing activity within the European Union (EU) countries such as Bulgaria, Greece and Romania is considered as one of the protected and promoted agro-industry The European commission conducts a protective policy for sericulture development by providing a direct subsidy in amount of EUR 133.26 for each silkworm egg box reared. The purpose is by sericulture development to provide additional livelihood of the people from less developed regions of Europe by the same time decreasing the unemployment and migration, to stabilize the rural economy and communities and to improve the conditions for work and life. From the European subsidy plus the cocoon purchasing price for only 3 months work it may be obtained an annual income by rearing 10 boxes of silkworm eggs in amount of around EUR 3000 which puts the sericulture into the most profitable agricultural crops. Sericulture development projects will be supported

by EU funds through the following measures of the Rural Development Program for the period 2007 – 2013:

- Professional training, informational activities and scientific knowledge dissemination.
- Establishment of farms by young farmers.
- Modernizing the agricultural farms.
- Value adding to agricultural and forestry products.
- Semi-subsistence farms.
- Creation of producers groups.

Direct financial support to the sericulture farmers in amount of US$ 8-9/kg fresh cocoons is provided by the Turkish government.

Considering the state subsidies to sericulture in some of the region countries, it may be concluded that their cocoons/raw silk produced are quite competitive at the market.

- Recently some of the biggest silk producers in the region such as Tajikistan and Uzbekistan attracted many foreign investors in their silk industry.
- The silk market in the region is in a process of development following the gradual increase of the people's living standard.
- Still available unique silk handcrafts production.

5. The solutions for future sericulture industries development

The BACSA region countries should use their unique advantages in sericulture development by means of product diversification. The main directions of the regional strategy for sericultural industries revival and development may be:

- Development of new sericultural products such as use the mulberry and silkworm for non – silk purposes. Utilization the rich germplasm and advanced science for development of new varieties, breeds and hybrids suitable for these aims.
- Considering the rich sericulture germplasm in Europe, an European mulberry and silkworm genetic resources preservation center may be established in one of the member states through the EU financial support programmes. The main tasks of this center could be preservation of all mulberry and silkworm varieties available in the member states in parallel with the countries of their origin, development of innovative techniques for the germplasm preservation, supply of the member states with genetic material for research purposes etc.
- The BACSA countries may be established as sources of sericultural genetic resources for the developing countries which may be paid by the respective donor organizations.
- Production in bi/multilateral cooperation mulberry sapling of highly productive varieties and high quality silkworm eggs for meeting the local needs and for export as well.
- Production of very high quality bivoltine silk with certain special characteristics. As per the advice of Mr. E. Seitz, a long-term silk textile expert if the BACSA countries are able to offer grey silk woven fabrics,

mostly “Creep De Chine”, 60 m without fault, alternatively in 90 cm and 150 cm width they could be highly competitive. Since the BACSA region is within or nearby EU if these fabrics are produced on stock the BACSA countries would have the advantage to deliver them within days to Italy and France. With modern automatic machines, wages would not play a major role. The looms would weave the same product all over the year, no change in yarn count, no change in the loom, no change of quality, thus such plant would be extremely competitive.

- Silk handcraft production for selling to the tourists at the local market and also for export.

The BACSA region countries could offer the following fields for cooperation and business partnership with the R & D institutions and the silk industry from other countries:

- Supply with high quality P_1 and F_1 bivoltine silkworm eggs;
- Making exchange of mulberry varieties and silkworm hybrids;
- Participation in joint research projects, financed by the EU and/or other donors;
- Short/medium term training courses of trainees from BACSA region in other countries and vice versa;
- Conducting bachelor, magisterial and PhD training courses of foreign students in some BACSA countries;
- Establishment of joint venture companies for production of raw silk, fabrics and garment;
- Establishment of joint trading companies for sell of silk products in Europe.

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Research concerning Durable Management and Integrated Production in a Family Reproduction Sericultural Farm.

Alexandra Matei[1], Agatha Popescu[2], Viorica Sladescu[3], Maria Dan[5], Magda Androne[1], Marilena Talpes[4], R. Radulescu[5].1Commercial Society SERICAROM-Research department Bucharest, Romania, 2University of Agricultural Sciences and Veterinary Medicine Bucharest, Romania, 3National University of Fine Arts Bucharest – Textiles Arts Department, Romania, 4Institute of Research and Development for Aquatical Ecology, Fishing and Aquaculture Galaţi, Romania, 5National Institute of Research and Development for Textiles and Leather Goods Bucharest, Romania.

ABSTRACT

The paper presents a management system of the sericultural activities in family farms based on the principle of integration between the main activities, respective the production of reproduction biological material, with the processing of the waste products and the production of secondary products obtained from the main activity. The project is based on the long experience existing in sericulture in the country, on the high value silkworm races and hybrids, mulberry varieties and on the tradition in the domestic silkworm rearing and cocoon processing. Techniques and methods: specific for producing biological reproduction material (selection based on biological and technological criteria, pure breed reproduction or inbreeding), for achieving new textile structures of natural silk and other fibers (mohair, wool), for processing unreeling cocoons, for determining the nutritive value of the forage recipes and food conversion in body weight at fishes, for evaluating costs, incomes and financial results. The project can be applied in family farms, more exactly in households having at least 1 ha arable land (0.5 ha mulberry plantation and 0.5 ha mulberry nursery), 150 s.m. buildings as silkworm rearing houses and 2-3 family members dealing with the activities stipulated in the project. The main product is represented by silk cocoons. The yearly production capacity of the family farm is 400 kg cocoons. The cocoons can be partially used for obtaining silkworm eggs (1000 boxes) or can be sold to specialized reproduction units. Other cocoons could be processed in silk fibers and traditional handicrafts. The expectations for the average technical performances are the following ones: 6000 kg leaf yield production/0.5 ha plantation, 35 kg raw cocoon production/box, 400 kg raw cocoon production/0.5 ha mulberry plantation, 15 kg leaf consumption/kg cocoons, 80% pupation rate, 2.0 g raw cocoon weight, 0.400 g cocoon shell weight and 1200 m filament length. The expectations for the profit rate is 33.04% (1st variant) - 24.87% (2nd variant). The major project objective is to establish the scientific and economic fundamentals for setting up a family sericultural reproduction farm under the conditions of integrated production and management.

Key words sericulture, silk cocoons, silkworm eggs, mulberry saplings, handicrafts, economic efficiency

INTRODUCTION

During the last two decades, the most of countries where sericulture represents a traditional job are facing a deep decrease of cocoon and silk production. Known as an activity peculiar to rural areas and representing an

income source for farmers, sericulture is brought in the attention of Governments whose efforts are focused to recover this field of activity.

At the world level, sericulture is practicing in farms of various types and management.

A mid-term and long-term national programme set up in India provides, among other measures, the joining of sericultural farms in order to allow extension services and to apply modern technologies. Also, another purpose is the creation of sericultural associations between raw material producers and users (reproduction cocoon producers-seed producers; cocoon producers-spinning mills; spinning mills-weaving mills) (Dandin, S.B., 2002).

In Thailand, a new trend in sericulture management has been noticed. Sericultural farmers are joining by activity type: young larvae rearing, cocoon producing (Chuprayon S. and coll., 2002).

The organization of farms of various production capacity where sericultural activities are combined with other activities (pig growing, cattle farming, mushroom growing etc) are suggested by Petkov N. and coll. (2004).

Taking into account the local conditions in Bulgaria, Grekov D. and Tzenov P. (2005), consider that the strategy for a short and mid-term sericulture rehabilitation has to adopt two farm types as follows: (a) 0.2 ha mulberry plantation, 30 s.m. rearing space, 150-200 kg annual raw cocoon production; (b) 0.5 ha mulberry plantation, 75 s.m. silkworm rearing house, 450-500 kg raw cocoon production.

A series of technical and management measures have been taken in order to encourage sericulture rehabilitation in Greece (Kipriotis E., 2005), Ukraine (Galanova O.V. and coll., 2005), Turkey (Karagozoglu A., 2005), Uzbekistan (Homidy H. and coll., 2005). Most of the authors mentioned above have pointed out the importance of cocoon processing in a traditional system in order to achieve traditional silk handicrafts, but also other types of commodities where natural silk is combined with other fibers, competitive both in the domestic and international markets.

During the last years, in the majority of the sericultural states, a special attention has been paid to wastes and sericultural secondary products (vegetal remains and excreta, pupae, unspinning cocoons, wastes coming from cocoon spinning (Kang S.R. and coll., 2002; Zi Ran Huang, 2002; Popescu A., Matei A., Sladescu V., 2007).

In Romania, sericulture has been practicing for years both within peasant households rearing silkworm without using any specific technical endowment and within state sericultural farms specialized in silk cocoon production, destined to cottage industry. The state farms were well endowed with 50-100 mulberry plantations and rearing buildings where 20-50 ton cocoons were achieved. The last type of production farms was not effective, as large sized farms were not suitable to the seasonal character of sericultural production and not able to assure permanent employment as well as due to the lack of integrated activities.

At present, the state farms are dissoluted as land has been given back to the old owners, spinning and weaving mills processing cocoons in natural silk products were closed. Under these circumstances, sericulture development has to be approached in a different way.

Within this framework, research and development in the field of sericulture will be running between 2005 and 2008 within a project destined to the rehabilitation of sericulture in Romania based on a new concept.
The project aims to recover silkworm rearing in Romania and is based on the experience existing in the country in this field of activity, but also on the existence of high value biological material represented by silkworm races and hybrids and mulberry varieties, rearing technologies and a long tradition in silk processing in handicrafts.

The project objectives are the following ones:

- Organization of a pilot sericultural farm endowed with the corresponding investment needed for running the basic activity (reproduction biological material) and marketing of the secondary products resulting from the basic activity;
- Economic optimization of the reproduction farm by the use of secondary products;
- Setting up the technology for producing the reproduction biological material (silk cocoon and mulberry seeding material) for obtaining new types of natural fibers, for achieving a new type of forage for aquaculture.

MATERIALS AND METHODS

The activities which will be running within the project are based on the following:

- Methods and techniques specific for producing biological reproduction material (selection based on biological and technological criteria, pure breed reproduction or inbreeding);
- Traditional methods and biotechnologies for multiplying the vegetal sericultural genetic stock (Morus sp.) represented by cutting, chinese layering, seeding;
- Specific techniques for achieving new textile structures of natural silk and other fibers (mohair, wool) with a novelty character;
- Textile techniques and technologies for processing, painting, weaving, knitting, finishing the raw material from textile wastes;
- Methods for determining the nutritive value of the forage recipes and food conversion in body weight.

The analysis of the economic results of the experiment variants will be made by the comparison method among variants (profit rate).

The following activities will be achieved within the project:

Activity 1. Silkworm rearing for cocoon producing with the following use:

1st Variant:

- 300 kg cocoons (75% of annual production) for producing 1.000 boxes of silkworm eggs;
- 100 kg cocoons (25% of annual production) for producing fiber and fabrics in the cottage industry;
- 15 kg pupae used as a component in fodder recipes for fishes set up within this project.

2nd Variant:

- 300 kg cocoons are sold to the egg producing units;

- 100 kg cocoons are processed in fibers used for handicraft producing;
- 300 kg dried pupae are used for producing fodder for fishes.

Activity 2. Producing mulberry seeding material

Using 0.5 ha land surface, according to the applied technology, 50.000 mulberry saplings or 5.000 layerings are expected to be achieved. Mulberry saplings are destined to be sold in the market.

Activity 3. Silk cocoon processing in handicraft industry

It is followed the activity of producing traditional silk handicrafts, using natural silk in combination with other fibers. The fiber combination and its processing technology will be established along the project running.

Activity 4. Sericultural By-products processing

Pupae, excreta and mulberry leaves could be used for producing fodder for fishes, based on specific recipes set up within this project.

Activity 5. Management of family reproduction farm under the condition of integrated production

a) Management of family reproduction farm under the condition of integrated production between mulberry tree culture , silkworm rearing and hand-made processing of sericultural waste;
b) Comparative evaluation of profitability for the experimental combinative integration variants in order to offer alternative and viable solutions for increasing economic and financial performances, profitability and competitiveness in sericultural farms.

The material used for the activities and variants mentioned above is represented by:

- Silkworm eggs (P1) – 12 boxes distributed for the two rearing series as follows: 8 boxes for Spring series and 4 boxes for Summer-Autumn series;
- Mulberry leaves (6.000 kg) obtained from 0.5 ha plantation;
- Mulberry seeds (10 kg) producing 50.000 mulberry saplings in a 0.5 ha plantation;
- Sericultural equipment;
- Special equipment for silk cocoon processing in handicrafts;
- 150 s.m. rearing space, organized within the existent buildings (houses temporary destined for this purpose, sheds, storehouses etc) or could be built new rearing houses. In both cases, it is required to assure the corresponding arrangements imposed by the growing technology. A part of these spaces could be also used as a weaving mill in the simple variant, handicraft industry.

EXPECTED RESULTS

Technical performances

In a family farm, endowed with:

- 0.5 ha mulberry plantation
- 0.5 ha mulberry nursery
- 150 s.m. rearing space

- Specific sericultural equipment
- Equipment for the traditional processing of silk cocoons in handicrafts.

The following technical performances are expected: mulberry leaf production/0.5 ha-6.000 kg; silk cocoon production/box-35 kg; silk cocoon production/0.5 ha-400 kg; leaf consumption/kg cocoons-15 kg; pupation rate-80%; raw cocoon weight-2.0 g; silk shell weight-0.400 g.

Financial performances

In order to carry out the planned activities, the following indicators have been estimated:

- Total investment: 12.000 Euro, with annual quota of 3.000 Euro;
- Annual operating costs 32.120 Euro (1st variant) or 30.950 Euro (2nd variant).

The products sale will result the following incomes: 46.725 Euro (1st variant) or 42.394 Euro (2nd variant).

The expected profit is: 11.605 Euro (1st variant) with 33.04% profit rate and 8.444 Euro (2nd variant) with 24.87% profit rate.

Social impact of the project

- Creation of new vacancies and maintaining the rural population in villages;
- Reduced risk for environment polluting;
- Energy low consumption;
- Natural silk is an ecological product, consumers prefer natural products.

Cultural impact of the project

- The creation revival of traditional handicraft within the peasant household;
- Improving consumer taste and refinement.

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Sericulture in Greece and in the European Union
Facts of today and Prospects for tomorrow

Marios Tzitzinakis (*)[1, 2], Paschalis Harizanis [2, 3] and Antonios K. Perdikaris [4]

1 : *Hellenic Ministry of Rural Development & Food, Directorate of Animal Production, Department of Apiculture – Sericulture*
2 : *Sericultural Laboratory of Athens*
3 : *Agricultural University of Athens, Laboratory of Sericulture & Apiculture*
4 : *Permanent Representation of Greece to the European Union*
*** :** To whom correspondence should be addressed:
- **Sericultural Laboratory of Athens. 75 Iera Odos, 118 55, Athens, Greece.**
- **Tel.: 0030 210 5294565**
- **Fax: 0030 210 3466692**
- **E-mail: tzitzi1972@yahoo.gr**

ABSTRACT
Silk production and all the related activities, have a long tradition in Greece, as it had been one of the most profitable sectors of the agricultural economy of our country in the previous years. Today there is an effort being made, for the revival of sericulture in Greece. Among the European Union Member States, Greece seems to be the one that can play the most significant role for the development of the sector in Europe, since it is the country with the greatest cocoon yield per year. Furthermore two cocoon reeling plantation with maximum capacity that can cover the production of the whole area are located in the northern and central Greece and a silkworm egg production institute can produce eggs suitable and acclimatized for the condition of Greece and its neighbor countries.

Introduction

Silk production and all the related activities (silkworm rearing, silkworm egg production, cocoon processing, fabric production etc.) had been familiar for all the Greek territory since very early periods and among the most profitable sectors of the agricultural economy of our country in the previous years.

The period from 1908 until 1928 could be considered as the "golden period" of Greek sericulture. During that period, dry cocoon production reached high levels and after 1925 stayed continuously around 1.000 tons per year, reaching its peak in 1934, with a production of 1.149 tons of dry cocoons. Furthermore, it is mentioned that the fresh cocoon production in Greece, the year 1938, was 3.489 tons. Silkworm egg production as well, started increasing and reached a level of 145.000 boxes of 25 g each, per year, a big part of which was exported (1, 2).

Today there is an effort being made, forthe revival of sericulture in Greece, an objective which has partly been accomplished. It is believed that the production and processing of high quality silk, like the one which Greek breeders can produce, could be an alternative solution against the problems that many farmers are facing nowadays. The greatest interest for silkworm rearing in Greece, nowadays, is observed in the regions of eastern Macedonia and Thrace. A lower range interest for silkworm rearing appears in some other areas also.

The current situation in Greece and in the European Union

The districts where silkworms were reared in 2007 in Greece were: **i)** Southern Evros (Alexandroupolis – Soufli), **ii)** Northern Evros (Orestiada), **iii)** Serres,**iv)** Rodopi (Komotini), **v)** Kavala, **vi)** Magnisia (Volos), **vii)** Viotia (Tanagra), **viii)** Evia, **ix)** Lesvos and **x)** Hania (Crete Island). Most of those areas are on the northeastern part of the country (3).

The silkworms were successfully reared, according to the Eu ropean Union (EU) Regulations and the Greek Legislation (4, 5), in a percentage that ranged between 36,71% and 100%, per district, whereas in two cases, Magnisia and Viotia, the percentage of the success of the rearings was zero, since the yield of wet cocoons was less than 20 Kg per box of silkworm eggs used.

The precise data concerning the production of wet cocoons, the number of the silkworm egg boxes that were subsidized and the mean yield per egg box reared in the EU Member States, per country for the years 1995 - 2007 are presented on **tables 1 – 3** (6).

Table 1: Production in Kg of wet cocoons per country in the EU (European Commission DG AGRI, C.1)

	France	Italy	Greece	Spain
1995	1.390,0	15.047,0	22.000,0	205,0
1996	1.224,0	22.946,0	17.661,0	160,0
1997	1.763,0	21.671,0	24.065,0	410,0
1998	1.215,0	26.997,0	32.982,0	520,0
1999	1.375,0	38.189,0	30.238,0	760,0
2000	1.058,0	60.795,0	19.771,0	1.093,0
2001	665,0	57.177,0	37.040,0	996,0
2002	1.163,0	19.980,0	41.225,0	1.649,0
2003	1.037,0	754,0	48.938,0	1.204,0
2004	1.004,5	3.332,0	59.737,0	1.239,0
2005	899,0	2.763.0	73.889,0	1.662,6
2006	716,0	4.840,0	89.119,0	1.698,0
2007	918,0	5.581,0	103.579,0	1.712,0

Table 2: number of reared silkworm egg boxes which were subsidized in the EU (European Commission DG AGRI, C.1)

	France	Italy	Greece	Spain
1995	54	618	1.064	10
1996	55	1.020	868	8
1997	59	1.070	1.138	19
1998	54	1.312	1.455	26
1999	56	1.892	1.531	37
2000	49	2.896	1.012	47
2001	31	3.011	1.841	42
2002	50	2.154	1.961	73
2003	48	96	2.346	53
2004	43	150	2.795	55
2005	41	129	3.277	73
2006	34	228	4.215	74
2007	42	263	5.434	71

Table 3: Yield per egg box reared in Kg of wet coons (European Commission DG AGRI, C.1)

	France	Italy	Greece	Spain
1995	25,70	24,30	20,70	20,50
1996	22,30	22,50	20,30	20,00
1997	29,90	20,20	21,10	21,60
1998	22,50	20,60	22,70	20,00
1999	24,60	20,20	19,80	20,50
2000	21,60	21,00	19,50	23,30
2001	19,50	19,00	20,10	23,70
2002	23,30	9,30	21,00	22,60
2003	21,60	7,90	20,90	22,70
2004	23,09	22,20	21,40	22,53
2005	21,93	21,21	22,55	22,78
2006	21,06	21,23	21,14	22,94
2007	21,86	21,22	19,06	24,11

The unfavorable environmental conditions that were noticed in Greece in the begging of the summer of 2007, in connection with the delayed beginning of the spring rearings, due to problems in the procedure of the importation of the silkworm eggs from China, seem to have been the main reasons for the reduced yield per egg box reared in Greece, in comparison with the pervious years (7).

It is obvious from the official data, that among the European Union Member States with the greatest interest for sericulture, Greece seems to be the one that can play the most significant role for the revival of the sector. This assumption is confirmed by the fact that Greece itself has proven to be an ideal place for silkworm rearing and mulberry cultivation as far as concerns the climate and the environmental conditions in general, having demonstrated a long

tradition in sericulture and what's more, several other countries at the area, as Turkey, Bulgaria, Romania, Egypt are familiar with sericultural activities.

In addition to the previous remarks, in Greece recently relived the interest for producing silkworm eggs for commercial use, after a long time period during which it had been arrested, under the pressure of the problems that were observed, concerning the quality and the adaptation of the hybrids that were imported, in connection with the difficulties during the procedure of the importation. The produced eggs are monitored and certified for their quality and their hygiene condition by the Sericultural Laboratory of Athens. The same laboratory has also provided the silkworm egg producer with the raw material (8, 9, 10), that is some of the pure lines that have been preserved for many years, in order to develop the Greek commercial hybrid **X1 x K1**. This hybrid had been tested for several years in laboratory conditions and in the field, before beingprovided to the farmers and it showed remarkable characteristics and adaptation to the Greek conditions.

The amount of silkworm eggs of the Greek commercial hybrid **X1 x K1** that were produced and used by the Greek farmers the previous years was 1.300 boxes of 20.000 eggs per box for 2004, 3.000 boxes for 2005 and 3.500 boxes for 2006 (3). During those three sericultural periods almost all the farmers who reared that new Greek hybrid were satisfied with the yield, its resistance to diseases and the adaptation to the climatic conditions of Greece (11, 12, 13, 14, 15, 16) For the sericultural period of 2007, about 3.500 boxes of **X1 x K1** eggs were produced, inspected and certified by the Sericultural Laboratory of Athens (17, 18).

Another very critical factor affecting the sericulture development is the cocoon processing industry. Two plantations of silk reeling and yarn production are located in Greece. The one is at the district of Evros (north-eastern Greece) and the other at the district of Viotia (central Greece). The total amount of the Greek production and a great part of the yield of the neighbor countries could be absorbed by the above mentioned plantations, if they operated at their maximum capacity. The operation of these two factories could affect the cocoon prices given to the farmers and develop conditions favourable for expansion of the sericultural activities.

Support of the sector in the E.U.

Recently took place the incorporation of the European Regulations concerning sericulture in the integrated document about the establishment of common organisation of agricultural markets and on specific provisions for certain agricultural products (5). In section VII, article 111 of Council Regulation (EC) No 1234/2007, is mentioned that:

"*Aid shall be granted for silkworms and for silkworm eggs reared within the Community. The aid shall be granted to silkworm rearers for each box of silkworm eggs used, on condition that the boxes contain a minimum quantity of eggs, to be determined, and that the worms have been successfully reared. The aid per box of silkworm eggs used shall be EUR 133,26*".

Furthermore, in Commission Regulation (EC) No 223/2008 laying down conditions and procedures for the recognition of producer organisations of silkworm rearers (19), is mentioned that ***"Member States shall recognise producer organisations of***

silkworm rearers" and that **"*in order to obtain recognition, producer organisations must include at least 50 producers who use or undertake to use at least 2.500 boxes during the marketing year in which recognition is granted*"**, on contrary to the repealed Regulation (EEC) No 822/76, which mentioned that at least 500 producers were necessary to obtain recognition. This modification was adopted after a suggestion of the Greek Services and the Permanent Representation officers.

Conclusions

The adoptation of the recent Regulations that were mentioned above indicates that E.U. tents to support the sector of sericulture. The increasing demand within the European countries for natural and biological products and the increase of silk products consumption, which is steadily growing in Greece and in Europe, is mainly covered by imports. This fact creates opportunities for further development of sericulture and silk product processing in Greece and other European countries, with tradition and experience in the sector. Both silkworm rearing and mulberry cultivation, within the European Union countries are subsidized.

The rational exploitation of the subsidies in connection with the use of the well adapted local silkworm populations and hybrids, which can produce a very competitive by means of quality product, could be the critical factors that can assist sericulture regain its position in the agricultural economy of Greece and other European countries. Moreover, encouraging the local production of silkworm eggs could ensure the constant inspection of their production and conservation process and guarantee the availability of the required amount of eggs on the proper time, according to the climatic conditions of each year in connection with development of the mulberry leaves.

The capability of cocoon processing that can be provided to the Greek farmers, in the case of operation of the two existing reling plantations, can also ensure to the silkworm rearers a reasonable income.

The greatest competitor against the Greekand European silk industry is the low cost silk products that are imported from Asian countries, due to low cost labour that is provided on these areas. The improvement of the quality of the European silk products according to the needs of fabric industry and haute couture through the utilization of local genetic resources, the total exploitation of all the potential by-products and the implementation of innovative rearing systems for decreasing the production cost (20, 21), are some of the most essential factors for the sustainable development of European sericulture and silk industry.

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REALIZATIONS AND PERSPECTIVES FOR SERICULTURE IN ROMANIA

Elena Pau, Marilena Constantinescu
C.S. SERICAROM Research Department, Bucharest, ROMANIA

Romania is located in south-east Europe, having favorable climatic conditions for mulberry cultivation and silkworm rearing.
Sericulture – traditional activity in Romanian culture, is one of the oldest branch of agriculture.

ORGANIZATION AND MATERIAL RESOURCES FOR ROMANIAN SERICULTURE

The research sericulture activity and production of vegetal and animal biological material in Romania is organized by C.S. SERICAROM Research Department.
Even if Romania had a production of 1800 tons raw silk cocoons in 1989, nowadays the production is about 5 tons cocoons. This situation can be explained by the transition period of our country and by the damage of cocoons gathering activity and silkworm eggs distribution's network.
Today, according with the legislation, C.S SERICAROM is changing into BANEASA STATION FOR SERICULTURE RESEARCHES, public institution, who will coordinate the activities regarding research and production of vegetal and animal biological material.

MULBERRY CULTIVATION

The most popular mulberry species from Romania is Morus alba. Hybrids and local varieties: Calafat, Galicea, Basarabi, Eforie are highly appreciate and cultivate into rural areas. The genetic potential of these varieties ensure a production of 10-12 tons of leaves/ha with 24% protein content.
The vegetal genetic fond contents 59 Romanian and foreign mulberry species: Japanese, Chinese, Italian, Russian, Bulgarian.
The mulberry plantations from our country are presented as low bush, medium trunk and high trunk. These types of plantations are realized by the cuttings applied to mulberries during the first year after plantation. 2.5 -3 m distance between lines and 0.5 – 3 m distance in line provide a density of 1100 – 8000 plants/ha.
The genetic potential of mulberry varieties cultivated in Romania is able to cover the silkworms necessary leaves in order to obtain about 300 kg cocoons/ha. The mulberry multiplication methods are: cuttings, graftings, also by seeds.

VARIETIES AND SILKWORM HYBRIDS. SILKWORM EGGS PRODUCTION

In Romania, there are reared only silkworms for mulberry leaves (Bombyx mori L.). We have 75 local and foreign silkworm races into the genetic fond of Bombyx mori species: Japanese, Chinese, Russian, Bulgarian, Indian. During the breeding program there were obtained hybrid lines with high genetic potential.

The medium cocoons' weight is 2.0 – 2.5 g, for cocoon silk shell is 0.430 – 0.490 g and the fiber length is between 1100 – 1400 m.

In our country, it can be produced silkworm eggs on demand, for local consumption or exportation.

THE SERICULTURAL PRODUCTS PRICES

The present prices are:

- 20000 silkworm eggs/box …….… 7 euro
- cocoons, I^{s} quality..... 5 euro/kg
- cocoons, IInd quality.....2 euro/kg
- young mulberry species.....2 euro/each
- young mulberry hybrid.....1 euro/each

From 2005, The Ministry of Agriculture and Rural Development gave a subvention of 0.8 Euro/kg for cocoons, if the rearer obtained a production of 20 cocoons kg/silkworm eggs box. Starting with 2008, The European Union is offering, for the same conditions, a subsidy of 136.26 Euro/box.

THE RESEARCH ACTIVITY IN SERICULTURE

The research activity in sericulture in carried on by The Academy for Agriculture and Forestry Sciences.

The main activity areas are:

- silkworm and mulberry breeding;
- mulberry plantations maintenance and exploitation;
- silkworm rearing technology;
- silkworm and mulberry diseases.

The main topics studied by the research stuff are:

- preservation of silkworm and mulberry genetic resources; experimentation of new methods in this field;
- elaboration of mulberry preservation programs, based on morphological, physiological and biochemical criteria;

for silkworms the main criteria being silk fiber length and food utilizations

capacity;

- the use of informatical systems in order to form the data base needed to elaborate new breeding programs;
- the study of biometric mulberry parameters, as expression of eco pedological conditions for different areas;
- biotechnological methods used for mulberry multiplication based on cells cultures;
- study of silkworm and mulberry pests; elaboration of control and disease prevention;

- obtaining of sericulture by-products: silkworm eggs' EXTRACT EMBRIONAR – bio energizing nutritive supplier for endocrine system and sexual functions;
- the oil obtained from chrysalis it can be used in cosmetics, silk powder; used into leather industry; mulberry fruits may be used in food industry, proteic extract from mulberry leave may be used in food industry and cosmetics.

Between 2002 – 2007 were in progress 20 research programmes, financed by National Research Programmes. The research results were transferred in practice, using a World Bank Programme.

SERICULTURE REORGANISATON SOLUTIONS IN ROMANIA

Having in mind the Romanian agriculture situation during the transition period, the sericulture specific characteristics for the same period were the following:

- lower cocoons production and as consequence, the percentage of silk fiber from total fibers was decreasing;
- abolishment or reorientation of some sericulture production units;
- lack of natural silk reeling machines;
- lack of specific legislation and promotion on a governmental level.

In these conditions, the main objectives on medium term strategies for sericulture are:

- to set right the sericultural production, high standards for fiber quality, which substantially influence the silk price into the European Union;
- resize of sericulture exploitations, their modernization and new technologies;
- attraction and motivation for individual rearers to open small sericultural farms;
- sericulture production diversification through distinguishing secondary sericulture products;
- development of scientifically research, based on sericulture production demand and promotion of technology transfer;
- development of marketing activity in sericulture.

The sericulture activity represents an important source for additional income in the rural communities, having a substantial role in quality life elevation, using human resources with less physical power.

The sericulture can be performed in some unfavorable areas from our country, where the climatic conditions allow it, knowing that mulberry plant – who represent the silkworm food – it can adapts to some areas with less favorable conditions.

Because of its specific, the sericulture protects and improve the environment, since in this activity there are not used pollution substances, the silk fiber being an ecological product, superior to all the other types of natural or artificial fibers.

By the renewing of sericultural activity it starts a traditional handicraft in Romania, the popular art products made from natural silk (headdress, national suits, etc) which are appreciated all over the world.

The sericulture relaunch in Romania is possible by organization the silkworm eggs distribution, cocoons gathering and primary processing into a private system or by Silkworm rearers professional association.
This new organizational concept for our country is applied with very good results in countries with traditional sericultural activity and who don't have an intensive sericulture activity, the individual system being efficient for this animal science branch.
The novelty for Romania would be made by some SME (small-medium enterprises) specialize for silkworm eggs incubation, silkworm young larvae rearing and distribution, cocoons gathering, primary processing (cocoons drying), obtaining silk fiber (into small reeling centers) and their commercialization on local and international market.
In order to have these objectives on SME level, there were made some financial paper work for:

- silkworm eggs incubation, Ist age silkworm larvae delivery toward rearers;
- silkworm eggs incubation, IIIrd age silkworm larvae rearing and distribution.

FINANCIAL DOCUMENTATION FOR SILKWORM EGGS INCUBATION; DELIVERY TOWARD REARER OF Ist AGE SILKWORM LARVAE

The silkworm biological cycle's activity should be realized by a private centralized system for village rearers or for an area of maximum 30 km.
The presented financial documentation is for 100 boxes silkworm eggs incubation into individual system, beingdelivered to rearers when they reach the *Ist* larval age (Annexes 1 and 2).
In this situation, is needed for rearer to have a surface of 200 square meters mulberry plantation surface.
The prices for larvaes in Ist age are in Euro, on a medium exchange rate of 3.2 lei.
It should be mention that the silkworm eggs value was included for this estimation. So far, in Romania the silkworm eggs are distributed for no charge without any legal prescription. And it's possible for the research stuff to do so because of the small distributed quantity.The activity is very profitable if from the estimation costs would be eliminated the human resources payment; in this situation, the enterprising income would be for about 215 Euro.

FINANCIAL DOCUMENTATION FOR SILKWORM EGGS INCUBATION; DELIVERY TOWARD REARER OF IIIrd AGE SILKWORM LARVAE

The private enterprises who want to have more services can extend their activity from silkworm eggs incubation, to rear them along two larval ages. For this activity, is needed 1000 square meters mulberry plantation.
The costs for this activity are presented into the Annexes 3, 4 and 5.
Analyzing the private enterprise activity, it can be observe that this activity produce a small income, who can be consider as an extra one, same for a silkworm rearer, who doesn't need too much work and workers.
If the private enterpriser endows himself with a cocoons drying furnace, he can obtain dry cocoon to get the silk fiber out of it.
The cycle would be complete with a reeling machine with 20 reeling heads, having the possibility to get on market with the raw silk fiber.

The business opportunities would be the textile industry by using the silk fiber, or for silkworm rearers, by usage of sericulture secondary products, who are well appreciate into chemical and pharmacological industry.
To develop cocoons production activity, it's necessary to subsidize the silkworm rearers, but also to encourage the small enterprises by having the possibility to get credits for small industry and by access to the European programmes.

Annex 1

General estimatefor 100 silkworm eggs boxes (s.e.b.)' hatching in a clasic household system (cardbord – 10 g s.e.b. capacity)

Nr crt	Specification	Work volume		Manual labor				Materials			
		MU	Total	Work norm	Total day/ man	Unitary price/ /day/ man	Total value lions	Name	MU	Quan-tity	Total value lions
1.	The hatching space mechanic cleaning	square meters	50	200	0.25	20	5	silkworm eggs	boxes	100	2400
2.	Sericulture equipment washing	square meters	36	150	0.25	20	5	desin-fection	liters	1	4
3.	Hatching space's whitewash with calcium hydro-xide $Ca(OH)_2$	square meters.	50	400	0.12	20	2.4	lime	kg	3	6
4.	The equipment and hatching space disinfec-tion; two times	square meters.	100	400	0.25	20	5	paper	kg	10	20
5.	Boxes manufactures	piece	100	400	0.25	20	5	Wor-ming			60
6.	The silkworm eggs setting on boxes in the hatching space	piece	100	400	0.25	20	5	other materi-als (% from costs)			40
7.	The hatching space microclimate superviseing	hours	50	8	6.25	20	150				
TOTAL					**7.62**	**20**	**152.4**				**2530**
TOTAL											**2682.4**

Annex 2
Technologic estimate
Phase – 100 silkoworm eggs boxes (s.e.b.)' hatching in a classic household system

Estimate's name/execution phase	Manual labor (lions)	Materials (lions)	Total value (lions)
100 s.e.b. in a classic household system	152.4	2530	2682.4
Total	152.4	2530	2682.4
Cost price/box	152.4	2530	26.82
Adding 20%/box	2682.4 : 100 boxes		5.36
Cost price/box			**32.18**

Cost price at 1.04.2008 ……..8,7 €/box
(1€ = 3,7 lions)

Annex 3
Technological estimate
Phase – young larvas rearing (y.l.r.) equivalent of 100 s.e.b.

Article no from normative	Specification	Work volume		Manual labor				Materials		
		MU	Total	Work norm	Total day/ man	Unitary price/ day/ man	Total value/ lions	Name	Quantity (Kg)	Total value/ lions
1.	The hatching space mechanic cleaning and whitewashing – 2 times	square meters.	300	600	0.5	20	10	- disinfection solution - lime - paper sheets - worming - other materials, % from costs (termometer, radiator, baskets, pails)	15	**30** **40** **60** **50** **25**
2.	Mechanic cleaning, buckling and shelves disinfection	piece	90	180	0.5	20	10		-	
3.	Shelves assembling, cutting and pileing up paper on shelves	piece	90	300	0.3	20	6.6		30	
4.	Larvas transfering from hatching boxes in rearing spaces	boxes	100	200	0.5	20	10		-	
5.	Maintenance work (feeding, silkworm larvas' sheets changing)	boxes	100	100/zi	12	20	240		-	
6.	Daily disinfections	piece	300	1500	0.3	20	6.6		-	
TOTAL					**14.1**		**282**			**205**
TOTAL										**487**

Annex 4
Estimate for larvae rearing, during the young ages from 100 s.e.b. hatching

Estimate's name/execution phase	Manual labor (lions)	Material (lions)	Total value (lions)
Young age rearing of silkworm larvas (I and II ages) from 100 boxes	282	205	487
Total	282	205	487
Cost price/box	487: 100 boxes		4.87
Adding 30%/box			0.97
Cost price/box			**5.84**

Annex 5
General estimatefor 100 S.E.B. hatching in a classic household systemand young age rearing of larvas

Estimate's name/execution phase	Manual labor (lions)	Materials (lions)	Total value (lions)
100 S.E.B. hatching in a classic household system	152.4	2530	2682.4
Young age rearing of silkworm larvas (I and II ages) from 100 boxes	282	205	487
Total	434.4	2735	3169.4
Cost price/box	3169.4: 100 boxes		31.69
Adding 20%/box			6.33
Cost price/box			**38.02**

Cost price at 1.04.2008 ……..10,27 €/box
(1€ = 3,7 lions)