NEW SILKWORM VARIETIES OF PAKISTAN

BY

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Abstract

Silk seed is the basic requirement for silk production. This article describes the work of silkworm breeding in Pakistan. Four new silkworm varieties were developed from imported F1 hybrid silkseed by in-breeding and selection to produce a F1 hybrid for commercial rearing in Pakistan. 1,500 boxes of F1 hybrid disease free silkworm seed was produced in 1988 and 2,100 boxes in 1989,for the first time in the country for distribution amongst the farmers for rearing. During breeding and selection of the bi-voltine races sex limited mutants were also selected for further research.

Introduction

Silk seed is the basic need for production of natural silk yarn. Silkworms are broadly classified as uni-voltine, bi-voltine and multi-voltine on the basis of their voltinism which signifies means number of generations of silkworms produced in a year under natural conditions. Bi-voltine races having short duration of development and strong constitution and high cocoon yield, are generally preferred for commercial rearing in countries of China, Japan and Korea. The parent stock of these races produced in these countries have been as their trade secret for last 1,000 years and they do not provide germ plasm to any their country in the world for breeding. Realizing the importance of this problem, research work was started in this regards in Pakistan Forest Institute, Peshawar, under an FAO/UNDP Project in 1984 with the guidance of a Silk Breeding Expert from Republic of Korea. Although egg breeding centers were established at Murree, Parachinar, Khanaspur and Rawalakot for breeding parental stock, long ago, but no significant remarkable achievements were made in their connection. Pakistan Agricultural Research Council also started work on evolution of races in 1982, but uptil now no commercial seed could be produced by them.

Lee, Park and Moon (1984) reported that 109 protein patterns in females and 112 patterns in males were found in 129 silkworm varieties. Yoshitake (1964) also moted that there is a distinct difference of electrophoretic protein, acid phosphatase and esterase patterns among the silkworm varieties. These results suggested that there is a possibility that electrophoresis can be used for identification of silkworm varieties to select pure lines.

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Under the prevailing conditions of Pakistan, it was observed that in the absence of any indigenous silkworm varieties, it will take very long time to evolve true parents. The studies were, therefore, undertaken to develop original parent races from imported F1 hybrid silk seed by the above method.

Method And Material

Silkworm rearing areas of the Punjab, Sindh and N.W.F.Province were surveyed for selection of few individual silkworm larvae for identification of racial characters of their parents by means of electrophoresis. The breeding was conducted continuously for purity of racial characters such as egg colour, larval marking, hatching ratio, cocoon shape and quality and molting habits until they become stable in a race. Selfing was made in second and third filial generation. The selection was made on individual basis to produce different breeding lines of Japanese and Chinese origins.

In the fourth filial generation for Chinese plain larvae, mixed rearing and individual selection were undertaken so that several lines as shown in Figure 1 can be made according to the genetic characters of egg colour and marking, cocoon shape and quality, and molting habits. Special attention was paid to the marking which was dominant as against the recessive plain larvae by keeping in view their hereditary characteristics. In the fifth filial generation, several strains appeared to be specific for egg colour, hatching ratio, molting habit, cocoon shape and quality in C-84-001-101 (Chinese strain).

At this stage, batch rearing and batch selection were made in order to identify whether they are pure or not. In sixth filial generation, the procedure was repeated to increase the number of homozygote individuals for easy selection.

The PFI-1 (given name of Japanese strain) and PFI-2 (given name of Chinese strain) are called the breeder's stock. Breeder's stock is called P3 which is reared for multiplication. For the maintenance of P3 only 20% of each batch was selected by weighing the larvae, cocoon shell and determining the cocoon shell ratio. The remaining population was discarded.

The grand parent stock (P2) obtained from P3 by multiplication was multiplied further at Pakistan Forest Institute, Peshawar to produce parent stock (P1). At this stage, about 50% of each batch was selected according to the standards. It is called P1 or parent stock.

Parent Stock (P1) obtained from P2 multiplication was reared at sub-station at Pateka (AJ&K) and multiplied further for getting large quantity of parent stock. At this stage, about 70% of each lot was selected by visual selection. Parents (P1) of both Japanese and Chinese origin were reared by the farmers to provide seed cocoons to the seed makers for F1 hybrid silk seed production by crossing.

After evolving pure lines, the germ plasm and the silk seed were preserved under controlled environmental conditions according to the standards prescribed for bi-voltine eggs. Performance trials of the evolved races for testing the adaptability to local climatic conditions were also conducted.

Incubation of parent silkworm eggs was undertaken by artificial hatching treatment. Under normal conditions, the hybernating bi-voltine silkworm eggs undergo a diapause stage in a year but Tanaka (1952), developed a method to completely break down the diapause of bi-voltine silkworm eggs by applying chemical stimulants at a certain stage of embryonic development to bring about normal hatching more than once in the same year. This method of rapid breaking of diapause stage of silkworm eggs and having more number of generations is known as artificial hatching.

The following standards of temperature and humidities were kept during incubation for bi-voltine parent eggs.

STANDARDS OF TEMPERATURE AND HUMIDITIES FOR NORMAL PARENTAL EGGS

No.of days		1	1	4	6
Temperature	^{0}C	15	18-20	23-24	25-26
Relative Humidity	%	80	80	15	80-90

STANDARDS FOR ARTIFICIALLY TREATED EGGS WITH HCL

No. of days		6	6
Temperature	^{0}C	24	25-26
Relative Humidity	%	75	80

Note: During last 5-6 days of incubation light exposure is increased by six hours per day

The procedure of developing pure lines from imported F1 hybrid is shown in Fig-1.

Fig.1 Diagram showing method of evolving pure lines from imported F1 hybrid silkseed.

F1

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F2

SELECTION OF INDIVIDUAL TO MAKE LINES-MARKING PLAIN (PLAIN AND MARKING), COCOON SHAPE AND COCOON QUALITY

CHINESE LINES (PLAIN)	!	JAPANESE LINES (MARKING)
LINE-A PLAIN	!	LINE-B MARKING

F3

CHINESE MIXED REARING AND INDIVIDUAL SELECTION		JAPANESE MIXED REARING AND INDIVIDUAL SELECTION
LINE-J-84-003-101-01	!	LINE-C-84-003-102-01
LINE-J-84-003-101-02	!	LINE-C-84-003-102-02
LINE-J-84-003-101-03	!	LINE-C-84-003-102-03
LINE-J-84-003-101-04	!	LINE-C-84-003-102-04
LINE-J-84-003-101-05	!	LINE-C-84-003-102-05

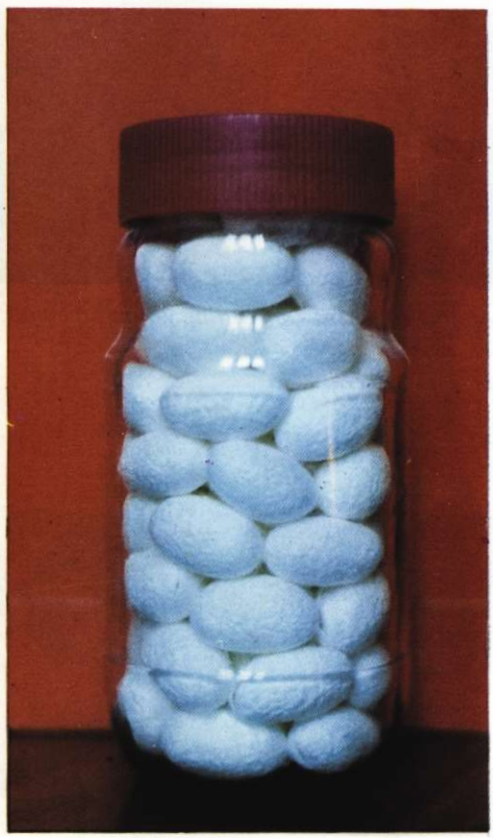
F4

CHINESE LINES BECOME PURE IN TERMS OF LARVAL CHARACTER AND ATTENTION NOW FOCUSED ON UNIFORM COCOON CHARACTER		JAPANESE BATCH REARING & INDIVIDUAL SELECTION
LINE-J-84-004-101-01	!	LINE-C-84-004-102-01
LINE-J-84-004-101-02	!	LINE-C-84-004-102-02
LINE-J-84-004-101-03	!	LINE-C-84-004-102-03
LINE-J-84-004-101-04	!	LINE-C-84-004-102-04
LINE-J-84-004-101-05	!	LINE-C-84-004-102-05

F5

LINE-J-84-005-101-01		LINE-C-84-005-102-00
LINE-J-84-005-101-02	BATCH REARING &	LINE-C-84-005-102-02
LINE-J-84-005-101-03	BATCH SELECTION	LINE-C-84-005-102-03
LINE-J-84-005-101-04		LINE-C-84-005-102-04
LINE-J-84-005-101-05		LINE-C-84-005-102-05

Japanes Race



Chinese Race

Chopping of leaves for feeding young silk worms.

feeding grown silk worms.

Feeding young silk worms

Larva teeding on leaves.

LINE-J-84-006-101-01 | LINE-C-84-006-102-01
LINE-J-84-006-101-02 | F6 | LINE-C-84-006-102-02
LINE-J-84-006-101-03 | BATCH REARING | LINE-C-84-006-102-03
LINE-J-84-006-101-04 | INDIVIDUAL | LINE-C-84-006-102-04
LINE-J-84-006-101-05 | SELECTION | LINE-C-84-006-102-05
|
F7
RACE REACHES PURE STAGE.

PFI-1 FROM LINE-J-84-006-103-04 | PFI-2 FROM LINE-C-84-006-104-04

PERFORMANCE TRIAL
WITH PFI-1 X PFI-2
|
AFTER AUTHORIZATION
|
CHINESE(P3) BREEDER'S STOCK (P3) JAPANESE(P3)
(AT P3 STAGE, 20 % SELECTION AT PFI)
|
P2
(AT P2 STAGE, 50 % SELECTION AT PFI)
|
GIVEN TO SEED MAKERS
FOR MULTIPLICATION
P1
(AT P1 STAGE, 70 % SELECTION AT PATEKA, AJK)
|
GIVEN TO THE FARMERS
FOR SEED COCOON PRODUCTION
|
COLLECTION OF SEED COCOONS
FROM THE FARMERS
|
F1 HYBRIDIZATION BY PFI & AJK
F1 (PFI-1 X PFI-2)
|
GIVEN TO THE FARMERS
FOR SILK PRODUCTION
AS COMMERCIAL SILK SEED

Results And Discussions

Selection:

Silkworm rearing was carried out to get pure lines from the imported F1 hybrid silk seed. The first generation was produced in autumn, 1984 in Peshawar. The following breeding lines were developed:

	Strain	No.of Eggs	Egg Colour
J-84-001-101	200	Purple	
C-84-001-102	324	Green	
C-84-001-103	423	Purple	
C-84-001-104	484	Green	

These eggs were preserved for different periods of time at different temperatures for diapausing.

08.11.84	-	18.11.1984	10 days at 25^0C
19.11.84	-	21.11.1984	2 days at 23^0C
22.11.84	-	26.11.1984	3 days at 20^0C
27.11.84	-	28.11.1984	2 days at 10^0C
Until starting incubation	95 days at 2-5^{o}C		

After hibernation, the incubation was started on 05.03.1985. The second generation was reared in spring, 1986 at Peshawar for further selection. The economic characters were as under:-

Strain	Average weight of Cocoon	Cocoon shell	Cocoon shell ratio
J-84-002-101-03	1.458	0.293	20.10
C-84-002-102-04	1.486	0.313	21.06
J-84-002-103-03	1.764	0.387	21.94
C-84-002-104-04	1.645	0.353	21.46

Due to high temperature in Peshawar, the third generation was reared at Parachinar in Spring 1985 after artificial treatment with HCL. The specific gravity of HCL was 1.075. The diapausing time was 5 minutes at temperature of 46.11oC. The specific gravity of HCl was 1.075. The dipping time was 5 minutes at temperature of 115 oF. The economic characters of the strains under study are as under:-

Strain	Average weight of Cocoon	Cocoon shell	Cocoon shell ratio %
J-84-003-101-03	1.316	0.285	20.60
C-84-003-102-04	1.403	0.311	21.27
C-84-003-103-03	1.530	0.376	24.58
C-84-003-104-04	1.528	0.330	21.60

For further purification, the fourth generation was reared in autumn 1985 at Pakistan Forest Institute, Peshawar with the following economic characters:-

Strain	Average weight of Cocoon	Cocoon shell	Cocoon shell ratio %
J-84-004-101-03	1.259	0.277	22.00
C-84-004-102-04	1.287	0.278	21.60
J-84-004-103-03	1.839	0.394	24.04
C-84-004-104-04	1.536	0.339	22.17

Rearing of fifth generation in spring 1986 was carried out at Institute, for selection and identification of both Chinese and Japanese parents. The economic characters of selected parents was as under:-

Strain	Average weight of Cocoon	Cocoon shell	Cocoon shell ratio %
J-84-005-101-03	1.924	0.487	25.31
C-84-005-102-04	1.407	0.327	23.24
J-84-005-103-03	1.544	0.374	24.22
C-84-005-104-04	1.602	0.389	24.28

Sixth generation was reared at Faisalabad in late autumn, 1986 (November-December), for further purification. Out of four strains, two strains reached pure stage according to the following standard racial character.
Racial characters of two authorized varieties:-

1. J-84-006-103-03(PFI-1) and C-84-006-104-04 (PFI-2)
(Japanese origin P_3) (Chinese origin P_3)

Egg Characters

Colour:..... PFI-1...Purple. PFI-2...Greenish, dark grey Discarded all the batches showing very light colour which were mostly unfertilized eggs.

No. of Eggs..... Discarded all the batches containing less than 300 eggs
Discarded all the batches having overlapped eggs.
Discarded all the batches having abnormal size eggs

Larval Characters

Ist Instar.....Dark and black color ants were kept.
Discarded all early and late molting larvae which constituted 20% of the total. Keeping 80% of uniform mollting larvae.

For making new strain, selected 10% early molting larvae from the batch to get early variety.

This practice was continued at least six generations to stabilize these molting characters to select early and late varieties.

2nd and 3rd instar....cream color. Discarded whole batch having 5-10% moves and not eating larvae. Microscopic examination was also done to see disease infection.

4th and 5th instar....

J-84-006-103-03...pinkish or brown color.
Marking ..crescent and star black.
Blood color...pinkish

C-84-006-104-04 ... White color
Plain...no marking
Blood color...white

Cocoon...

J-84-006-103-03... Peanut shape and white color.

C-84-006-104-04... Oval shape and white color.

The following two new varieties were evolved at the Institute, in Peshawar in spring, 1987. The racial characters are given below:-

Egg Color: PFI-3...Purple PFI-4...Greenish
Discarded all the batches showing very light colored and mostly unfertilized eggs.

No. of eggs... Discarded all the batches containing less than 300 eggs.

Discarded all the batches overlapped eggs.
Discarded all the batches having abnormal size eggs.

Larval Characters

Ist Instar... .dark and black color ants were kept.
Discarded all of early and late molting larvae(20%)
Keeping remaining 80% of uniform molting larvae.

2nd and 3rd instar...cream color.
Discarded whole batch having 5-10% larvae moving and but not eating. Microscopic examination was conducted for examination of diseases.

4th and 5th instar...
J-85-008-101-01...Pinkish or brown color.
Marking...crescent and star black.
Blood color...pinkish

C-85-006-102-02...White color
Plain...no marking
Blood color-white

-Cocoon...C-85-006-101-01...Peanut shape and white color

C-85-006-102-02...Spherical shape and white color

After final selection and purification, the racial and economic characters of 4 silkworm races are given in Table 1 below:

Table:1 CHARACTERISTICS OF AUTHORIZED PARENT STOCKS

	Hatch ability	Duration of larval stage	Fifth stage	Pupation ratio
PFI-1	90%	25 days 4 hrs	7 days 12 hrs	85
PFI-2	95%	25 "25 8	" 12 "	90
PFI-3	91%	25 " 16	8 " - "	86
PFI-4	92%	26 " 20	8 " 21 "	91

Larvae Marking	body color	Average Weight of cocoon	cocoon shell	Cocoon shell ratio
Marking	Redish	1.49	3.12	21.98
Plain	Bluish	1.773	4.26	24.02
Marking	Redish	1.810	4.02	22.21

Plain	Bluish	1.766	4.49	25.42

Cocoon Color	Cocoon Shape	No. of Eggs laid
White	Peanut	398
White	Spherical	497
White	Peanut	443
White	Spherical	503

It may be noted from the above table that the selected parents have more than 90 and 85% practical hatchability and pupation ratio, rspectively. The cocoon shells ratio varied from 21.98 to 25.42 percent which is quite higher than the international standard of (20%) of bi-voltine parents.

Hyberdization was conducted by crossing J-84-006-103-03 (PFI-1) and C-84-006-104-04 (PFI-2) to produce 100 boxes of F1 hybrid disease free silk seed for performance trials.

i) Characters of F1 hybrid silk seed of PFI-1 X PFI-2

The hybrid silk seed of J-84-006-103-03 (PFI-1) X C-84-006-104-04 (PFI-2) is suitable for rearing in both spring and the autumn. It was found that the young larvae grow and molt uniformly. This uniformity always makes rearing easy. However, special care should be taken to feed the silkworms sufficiently with fresh mulberry leaves, avoiding too high temperature at the stage of larvae. The body of larvae is blue-white in colour with normal marking. Cocoons are white in colour, short ellipsoidal in shape with adequate wrinkles on the surface. It has good cocoon yield and raw silk percentage.

ii) Characters of F1 hybrid silkseed of PFI-3 X PFI-4

The F1 hybrid of J-85-006-101-01 and J-85-006-102-02 can be reared in spring only. Uniform growth and molting always makes the silkworm rearing easy. This F1 is comparatively weak against diseases but has good appetite which results in more yield. The body of larvae is creamy in colour with normal marking. Cocoons are white in colour elliptical in shape with moderate wrinkles on the surface. This variety has good cocoon yield, very good reelability, long filament of cocoon and high percentage of raw silk.

The adaptability trials on these two hybrid varieties were conducted to study their performance before distribution to the farmers. The racial and economic characters of these two hybrid varieties are given in Table 2 below:-

Table:2 CHARACTERISTICS OF COMMERCIAL SILK SEED

Strain	Hatch ability	Duration Larval	stage	5th	Pupation instar	ratio
	%	Day	hrs	Day	hrs	
PFI 1 x PFI 2	95	24	10	8	5	87.2
PFI 3 x PFI 4	93	25	15	8	10	85.8

	Larva	Average Weight of			Cocoon	
Marking	Body color	a cocoon	cocoon shell	Ratio	Color	Shape
Marking	Bluish	1.631	3.74	22.93	White	Peanut
Marking	Bluish	1.883	4.52	24.00	White	Peanut

Sex limited mutant

Tazima (1941) discovered a translocation between W and the second chromosome in which W became marked by two noticeable genes (+p and pSa) for larval characters locating on the second chromosome attached to one end of W and concluded that W plays decisive role in sex determination.

The breeding lines which appeared to have sex-limited inheritance and obtained from the line,C-85-006-106 in 1986 are as follows:

Genotype	YMYM.....	Male with marking of larva
Genotype	YPYP.....	Male without marking of larva
Genotype	XMXM.....	Female with marking of larva

For the production of commercial eggs which are invariably hybrid silk seed, the male must be separated from the female at pupal stage by cutting cocoons before the emergence of months so that they may be mated only with authorized varieties. In case of sex limited strains sex-discrimination can easily be done any time soon after molting of 4th instar up to the mature larval stage.

The gene dominating the marking is tranlocated to the X chromosome which has close connection with female characters. If this marked female is crossed with male without marking, the resultant female larvae will always have marking, while the male larvae will have no marking.

Conclusions

Silkworm rearing was undertaken at departmental level in Azad Jammu & Kashmir with about 70 boxes of parental stock and about 600 kgs of seed cocoons were produced in 1988. Subsequently about 1,200 kgs of seed cocoons of both PFI-1 and PFI-2

were produced by the farmers from 130 boxes in Chenari (AJ&K) for production of F1 hybrid disease free silk seed in 1989 by crossing PFI-1 x PFI-2. Similarly about 2,100 boxes of F1 hybrid silk seed was produced and preserved at Khanaspur for commercial rearing by the farmers. It was found that these four new varieties were highly resistant to low humidity when reared at Parachinar in spring. Rawalakot was not found suitable place for parent silkworm rearing in July being rainly season.

After selection and purification, the Sericulture Department, Government of the Punjab and North West Frontier Provinces were provided 20 batches of germ of plasm each of Japanese and Chinese parental stock for the production of F1 hybrid silk seed. Nepal was also supplied 10 egg layings of PFI-1 and PFI-2 varieties.

For sex-limited inheritance, Development of a variety with sex-limited inheritance will take two more years for making them pure. After purification of this new variety sex discrimination will become easy and cost of labour employed for cocoon cutting will be saved.

For the promotion of sericulture in developing countries it is stressed that sericulturally advanced countries should provide their basic stocks for the use of breeding purpose or parental stocks to developing countries where cocoon production is being conducted as a cottage industry.

REFERENCES

Ahmed, M. I. and Park K. E. 1988 Silk seed production techniques. Brochure 2 (PAK/86/002 UNDP/FAO Special bulletin)pp:1-11.

Gamo, T. and. Ohtsuka Y. 1980 Phylogenetic studies on the racial differentiation of the silkworm, Bombyx mori, on the basis of polymorphic genes in hemolymph proteins. Bull. sericul. Exp. Sta. Vol. 28, No. 1:15-49.

Lee, S.M., Park K.E. and Moon J. Y. 1984 Studies on the varietal differences of the electrophoretic protein patterns in the pupal hemolymph of the silkworm, Bombyx mori. Korean J. Sari. Sci. 16(1), 35-51.

Takami, T. 1969 General Silkworm Eggs (in Japanese). Tokyo, Japan.

Takami, T. 1952 Silkworm Genetics (in Japanese). Tokyo, Japan.

Tazima, Y. 1972 Handbook in Silkworm Rearing. Fuji Publishing Ltd.Tokyo, Japan.

Tazima, Y. 1964 The Genetics of the Silkworm. Logos Press, Academic Press, London.

Yoshitake, N. and Akiyama M. 1965 Distribution of types of the blood acid-phosphatase in various strains of the silkworm, Bxmbyx mori. J. Seriult. Sci. Japan, Vol.7 34, No.2:99-103.