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[Review]

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Conservation of Multivoltine Silkworm (*Bombyx mori* L.) Germplasm in India - An Overview

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Indian multivoltine mulberry silkworm (*Bombyx mori* L.) strains are poor in silk productivity and fibre quality. However, they are commercially exploited for the past five decades either in the form of pure breeds or cross breeds because of their adaptability to adverse climatic condition and acclimatization to specific regions and seasons. In the present paper conservation strategies of multivoltine silkworm genetic resources are dealt along with detailed information on accessibility of genetic resources, method of genetic resources conservation, characterization of genetic resources for morphological and productivity traits of economic importance. Selection of best ten accessions based on various economic parameters including yarn quality and their scope for future utilization are discussed.

Key words: Multivoltine, Silkworm, Germplasm, Characterization, Conservation

Introduction

In India, traditional silkworm races are of multivoltine nature (Datta, 1984), which might have descended from common ancestor, presumably reared in the Quantong province in China and later spread along the coast (Krishna Rao, 1994). A great diversity of multivoltine must exist globally considering the fact that individual farmers are allowed to reproduce silkworm eggs throughout the tropical cocoon producing region (ESCAP, 1997). Though multivoltine races are poor in silk productivity and fibre quality, they are highly adaptive and their genetic constitution permits acclimatization to specific regions and seasons (Venugopalapillai and Krishnaswamy, 1987). Multivoltine strains digest poor quality mulberry leaf and still produce silk; and also they are relatively tolerant to diseases and adverse climatic conditions and environment.

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Evolution of productive multivoltine breeds in India

Untill the early part of 20^{th} century mostly pure races/ breeds were commercially exploited for silk production. The popular Indian traditional silkworm races are Pure Mysore from Karnataka, Nistari from West Bengal, Sarupat and Moria from Assam. C. nichi (Shi × nichi) a popular multivoltine race considered to be Japanese origin (Chatterjee *et al.*, 1993) and Pure Mysore reported by sourced from China during the regime of Tippu Sultan (200 years back) are widely exploited in South India for the silk production. Nistari (Vern: Savior) is being used in Gangetic plains of Bengal for more than 100 years (Mukherjee, 1898). Sarupat (Vern: Narrow leaf) and Moria are exploited along with Nistari in Brahmaputra valley. Similarly, Borapolu and Chotopolu are maintained in the North-East region like Assam, Meghalaya and Mizoram. Diazo and Rong Diazo of Chinese origin were widely exploited in India in the early years.

Tazima (1958, 1988) recommended that improvement of multivoltine silkworm races was possible only through hybridization with exotic races. Ghose (1949) attempted improvement of multivoltine Nistari by hybridization with Italian races, evolving breeds like Nistid, Ichot and Iton, but these hybrids did not gain popularity. From 1960, Central Sericultural Research and Training Institute (CSR & TI), Mysore evolved multivoltine breeds superior to Pure Mysore and Nistari using bivoltine as a male parent like Mysore Princes, Kollegal Jawan, Kolar Gold, TEP_2, NS_4 and Hosa Mysore. Similarly CSR & TI, Berhampore (West Bengal) evolved a number of multivoltine breeds *viz.*, A_4E, MBD_1V, MBDV, D_{146}, D_{30} etc. In 1981, CSR & TI, Mysore initiated a few breeding programmes to evolve multivoltine breeds with shorter larval period, higher silk content, disease tolerance and better silk quality, thus a few breeds like MY_I, RD_I, P_2D_I, B_2D and HS_2B were evolved by utilizing the indigenous races like Nistari, Pure Mysore, Sarupat etc. During 1986 - 89, productive multivoltine breeds like LMP, LMO, DMR and PO were isolated through line breeding approach by crossing female with larval markings and male without crescent marking of Nistari race (Krishna Rao, 1994). Later, Universities, Department of Sericulture (DOS) and other research organization evolved a number of productive multivoltine breeds for silk quality improvement and also to suit the regions and seasons specific needs (Datta *et al.*, 2001). In India, a large number of valuable silkworm germplasm of indigenous stocks like Kashmiri race, Ichon, Nismo, Nistid, Borapolu (*B. textor*), Chotopolu (*B. foraunatus*) and some other indigenous races were lost due to lack of proper germplasm management system within the country and mainly because they were not continuously used for commercial exploitation (Thangavelu, 1997).

Recently CSR & TI, Mysore evolved BL_{67} from multi × bi hybrid (HM × Nistari) × NB_7) and BL_{27}. BL_{67} was crossed with CSR_5, CSR_{19} and NB_4D_2 which resulted in new productive multi × bi hybrids *viz*., Sharavati (BL_{67} × CSR_5), Cauvery (BL_{67} × CSR_{19}) and Tippu (BL_{67} × NB_4D_2) which were found to be better than the popular hybrid Pure Mysore × NB_4D_2, this new hybrid (Cauvery) yield above 900 m filament length and produced 2A - 3A grade silk. Similarly, the Andhra Pradesh State Sericulture Research & Development Institute (APSSR & DI) evolved one multi × bi hybrid Swarnandhra (AP_1M × APS_8), which was found better than the popular Pure Mysore × NB_4D_2. Further, some more robust multi × bi hybrids (BL_{43} × NB_4D_2 and BL_{67} × CSR_{101}) were evolved for the silk production in the high temperature areas. Few disease resistant breeds were also evolved in multivoltine for resistant to $BmDNV_I$ such as C. nichi (non-diapause) and H_{330}.

For the purpose of sex separation in the hybrid egg production in grainages, a sizable number of sex-limited breeds were evolved. In late 1960, some of the sex-limited races such as Nistari (SL), $D_{14}b$ (SL) and MBD-1V (SL) were evolved at CSR & TI, Berhampore and AP_1 (SL) at CSR & TI, Mysore. Recently, few more sex limited breeds for cocoon colour were also evolved at CSR & TI, Mysore, they are PM (SL) and BL_{27} (SL) where female spin golden yellow colour cocoon and male spin greenish yellow colour cocoon; MR_I (SL) where female spins golden yellow and male spins white coloured cocoons.

Concept on conservation of seri-biodiversity

Conservation of bio-diversity is an issue of global importance being addressed in different fora in all parts of the world. Global attention on the problem of conservation of bio-diversity were first drawn at United Nations Environment Conference during 1972 at Stockholm which strongly advocated preservation of irreplaceable genetic resources for the prosperity of present and future generation. India is one of the 12 identified centres of origin of cultivated plants and mega bio-diversity centre. The wide ranges of ecological situation existing in the country and varied needs for domestic purposes have made it imperative to look back at our rich bio-diversity. Vavilov (1932) pointed out that, without coordination effort around, the valuable gene pool could be lost through negligence. In this context, the enormous wealth of genetic variation in the Indian traditional multivoltine resources has to be used judiciously in the current silkworm improvement programme and for catering to the future needs. The silkworm germplasm provide a wide genetic variation, a resource for breeding, which can be exploited in the breeding for evolution of new productive silkworm breeds. Conservation priority has to be goal and context dependent. In gene bank, individual variety serves as genetic resource to maintain breeds variability as a whole or to create variation in a new segregation of population subsequent to hybridization, this diversity once lost is impossible to recreate (Kumaresan *et al.*, 2004 a).

Strategies for management of multivoltine silkworm germplasm resources

Since silkworm genetic resources are widely utilized in experiments dealing with genetical, physiological, biotechnological aspects as well as breeding of new silkworm strains, Central Silk Board, the national organization responsible for the overall development of sericulture in the country rightly responded to the call of global biodiversity conservation programme and accordingly established an exclusive centre to conserve, augment and exploit two biological entities, namely Silkworm and Mulberry. As a result, Central Sericultural Germplasm Resources Centre (CSGRC) came into being in the year 1991 at Hosur (12.45°N, 77.51°E, 942 m above MSL; Temperature = Maximum: 27 – 36°C, Minimum: 14 – 19°C; Relative Humidity = Maximum: 56 – 90%, Minimum: 37 – 59% and Annual average rain fall = 800 – 1,000 mm), Tamil Nadu under the World Bank aided National Sericulture Project (NSP).

CSGRC was established with a specific mandate for collection, characterisation, evaluation and utilization of the silkworm genetic resources to facilitate silkworm crop improvement through breeding programmes. In India, it is estimated that a total of 196 multivoltine silkworm genetic resources are maintained in CSB research institutes, Government silk farm, Universities and other research insti-

Table 1. Name, parentage, origin and cocoon characters of sixtythree multivoltine silkworm germplasm

Sl. no.	Name of the breed	Parentage	Origin	Cocoon colour	Cocoon shape
1	PURE MYSORE	GR	KAR	Greenish yellow	Oval
2	SARUPAT	GR	ASM	White	Spatulate
3	MORIA	GR	ASM	White	Spatulate
4	TAMILNADU WHITE	$PM.J_{122}$	TNU	White	Spatulate
5	C.NICHI	GR	JAP	White	Dumbbell
6	HOSA MYSORE	$PM.A_4e$	KAR	Greenish yellow	Elongated
7	MYSORE PRINCESS	$(PM.NN_6D)$(Hosho. Shungetsu)	KAR	White	Oval
8	KOLAR GOLD	$(PM.NN_6D)$(Hosho. Shungetsu)	KAR	White	Oval
9	KOLLEGAL JAWAN	$(PM.NN_6D)$(Hosho. Shungetsu)	KAR	White	Oval
10	MY_1	PM.Nistari	KAR	LGr yellow	Elongated
11	P_2D_1	(PM.Daizo) NB_2C_1	KAR	Greenish yellow	Elongated
12	RONG DAIZO	Chinese hybrid	CHI	Greenish yellow	Spatulate
13	GUANGNONG PLAIN	Guangnong	CHI	White	Oval
14	OS_{616}	Oval, S_{15}	WBL	Yellow	Oval
15	RAJ	GR	BGD	White	Spatulate
16	G	N(X-ray), M_2, CB_1, KPG-B	WBL	Golden yellow	Oval
17	NISTARI	GR	WBL	Golden yellow	Spatulate
18	NISTARI(M)	Nistari	WBL	Golden yellow	Spatulate
19	NISTARI(P)	Nistari	WBL	Golden yellow	Spatulate
20	ZPN(SL)	Zebra(SL),Nistari	WBL	White	Spatulate
21	CB_5	N(M), M_2, KB, N_{122}, C_{110}, C_{124}, J_{124}	WBL	Golden yellow	Oval
22	KW_2	Nistari	WBL	White	Elongated
23	M_2	N(Chemical Mutagenesis)	WBL	Golden yellow	Oval
24	A_{23}	Nistari,M_2,O,G	WBL	Golden yellow	Oval
25	A_{25}	Nistari,M_2,O,G	WBL	Golden yellow	Elongated
26	OVAL	N(X-ray), M_2, KPG-B, CB_1	WBL	Golden yellow	Oval
27	O	Nistari (J_{122}.NN_6D.KA.KB)	WBL	Golden yellow	Oval
28	M_{83}(C)	M_{83}(Eur), Nistari	WBL	Golden yellow	Oval
29	B	Nistari, KPG-B	WBL	Golden yellow	Elongated
30	GNM	Guangnong	CHI	White	Oval
31	$A_{14}DY$	$(N_{122}.C_{110})(N_{124}.C_{124})N$	WBL	Golden yellow	Oval
32	A_4E	$(MYS_1.BW)(MYS(N_{122}.C_{110}, N_{124}.C_{124})$	WBL	Greenish yellow	Elongated
33	PA_{12}	$PM.A_4e$	KAR	Greenish yellow	Elongated
34	AP_{12}	$A_4e.PM$	KAR	Greenish yellow	Elongated
35	A_{13}	PM.KA	KAR	Greenish yellow	Elongated
36	PMX	PM(IRRD)	KAR	LGr yellow	Elongated
37	PMS_2	PM(IRR-FS)	KAR	LGr yellow	Elongated
38	MU_1	PM(IRRD)	KAR	LGr yellow	Elongated
39	MU_{11}	$PM.NB_{18}$	KAR	LGr yellow	Elongated
40	WAI_1	MUTN	MHA	LGr yellow	Elongated
41	WAI_4	MUTN	MHA	LGr yellow	Oval
42	MY_{23}	(HN.Nistari)(PM.HM)PA_{11}	KAR	Greenish yellow	Elongated
43	MW_{13}	$(PM.MP)NB_7$	KAR	White	Oval
44	MHMP(W)	(PM.HM)MP	KAR	White	Elongated

Table 1. Continued

Sl. no.	Name of the breed	Parentage	Origin	Cocoon colour	Cocoon shape
45	MHMP(Y)	(PM.HM)MP	KAR	Greenish yellow	Elongated
46	P_4D_3	(PM.Daizo)NB_4D_1	KAR	Greenish yellow	Elongated
47	NISTID(Y)	(Nistari.Italian race)	BGD	Golden yellow	Spatulate
48	NISTID(W)	(Nistari.Italian race)	BGD	White	Spatulate
49	NK_4	GR	THI	Yellow	Spatulate
50	CAMBODG	GR	CAM	Yellow	Spatulate
51	DAIZO	GR	CHI	Greenish yellow	Spatulate
52	LMP	(Nistari.Nistari M)	WBL	Golden yellow	Spatulate
53	DMR	(Nistari.Nistari M)	WBL	Golden yellow	Oval
54	LMO	(Nistari.Nistari M)	WBL	Golden yellow	Oval
55	MY_1(SL)	(AP_1 SL)MY_1	KAR	LGr yellow	Elongated
56	PM(SL)	(AP_1 SL)PM	KAR	LGr yellow	Elongated
57	BL_{23}	NA	KAR	Greenish yellow	Elongated
58	BL_{24}	NA	KAR	Greenish yellow	Elongated
59	MU_{303}	NB_{18} × PM	KAR	White	Oval
60	MU_{520}	KA × NB_{18}	KAR	White	Oval
61	MU_{10}	NA	KAR	White	Oval
62	TW × SK_6 × SK_1	NA	KAR	White	Dumbbell
63	SK_6 × SK_1 × TW	NA	KAR	White	Dumbbell

GR = Geographical race; KAR = Karnataka; ASM = Assam; TNU = Tamil Nadu; WBL = West Bengal; MHA = Maharastra; BNG = Bangladesh; THI = Thailand; PM = Pure Mysore; N = Nistari; HM = Hosa Mysore; M = Marked; P = Plain; W = White; Y = Yellow; CHI = China; JAP = Japan; CAM = Cambodia; LGr Yellow = Light Greenish Yellow; NA = Not available.

tutes other than CSGRC, Hosur. After removing the duplicates it is believed that approximately 100 multivoltine genetic resources are available for conservation. At present CSGRC maintains 63 multivoltine silkworm collection, which includes 53 indigenous collection, comprising 26 from Karnataka, 20 from West Bengal, 3 from Tamil Nadu and 2 each from Assam and Maharashtra and 10 exotic races, representing 3 from Bangladesh, 4 from China and 3 from Japan (Table 1).

Method of multivoltine germplasm maintenance

The silkworm genetic resources are collected in the egg stage from the different donor institutes, where it was evolved or maintained as a strains or breeding materials. During the process of collection, in order to avoid contamination of the main germplasm bank, the layings are disinfected with 2% formalin solution for 5 – 6 min and then washed with water before incubation. In general, the multivoltine races are reared many times in a year as they do not under go diapause. But, adopting cold preservation of eggs for 30 to 35 days at 5°C, five crops per year is followed. The collected layings need to be reared in isolation and thorough screening for diseases is conducted to ensure disease free condition before including them to the main stream.

Maintenance of silkworm germplasm

The major objective and task in germplasm maintenance is to maintain the true to type of individual collection. These collections are either pure silkworm breeds or geographical races. Pure breeds are generally uniform for morphological characters and show minimum variation for quantitative traits within the population. In contrary, geographical races show wide variation for morphological as well as quantitative traits. In both these cases, inbreeding depression should be avoided among the stocks. There is an increased probability that offspring will inherit the same genes from both parents when inbreeding is accompanied by the directional selection, the resulting families will be similar phenotypes and total genetic variance among them will be decreased. Hence, all races/strains are brushed as composite population to avoid such a course of inbreeding depression as well as to avoid genetic erosion and maintain the gene pool as far as possible. Composite laying is defined as collection of known number of eggs from a known number of individual laying sources that represents the whole population. In the case of *B. mori,* 40 disease free layings (dfls) are taken at random in each accession. The composite layings are prepared only after the body pigmentation takes place by taking approximately 50 eggs from each laying, 40 dfls are divided into two batches *i.e.*, 20 dfls in each batch. All the pieces from 20 laying sources are pasted on a slightly thick brown paper with paste and wrapped in white fine tissue paper after drying. Thus, each composite layings consists of 20 layings sources with about 1,000 – 1,500 individual eggs. Similarly one more composite laying is prepared from the rest of the 20 layings. Thus in each accession two composite layings are prepared for brushing, forming two replication, but each replication is from a different source. The replication is maintained for data collection and analysis of economically important quantitative and qualitative characters. For calculating fecundity, 5 individual layings are considered separately. The plan of stock maintenance for multivoltine resources is given in Fig. 1.

Long-term preservation of eggs of multivoltine silkworm genetic resources

The multivoltine silkworm accessions are reared many times in a year for their maintenance, as they do not undergo diapause. The continuous multiplication of germplasm not only increases the maintenance cost but also leads to genetic erosion. Current methods for maintaining these silkworm genetic resources require continuous subculturing, a costly, and time and space intensive activity. There is also some concern about the loss of unique strains due to environmental hazards, genetic contamination and changes in genotypes during the passage of generation (Takemura *et al.*, 2000). In China, two or three crop cycle for diapausing and non-stop rearing for non-diapausing multivoltine accessions are followed (Chen, 2002). In tropical zone of India, it is intricate to induce hibernation in the multivoltine accessions; and also the long term chilling affects the hatchability of the eggs, substantially besides the other growth parameters. It is therefore, imperative to work out suitable egg preservation schedule for multivoltine silkworm genetic resources. Moreover, development of long-term conservation methods is obligatory as the accessions are escalating with the introduction of new genetic resources over the years. To alleviate these shortcomings and risks, a method that would preserve the eggs safety at low temperature for a

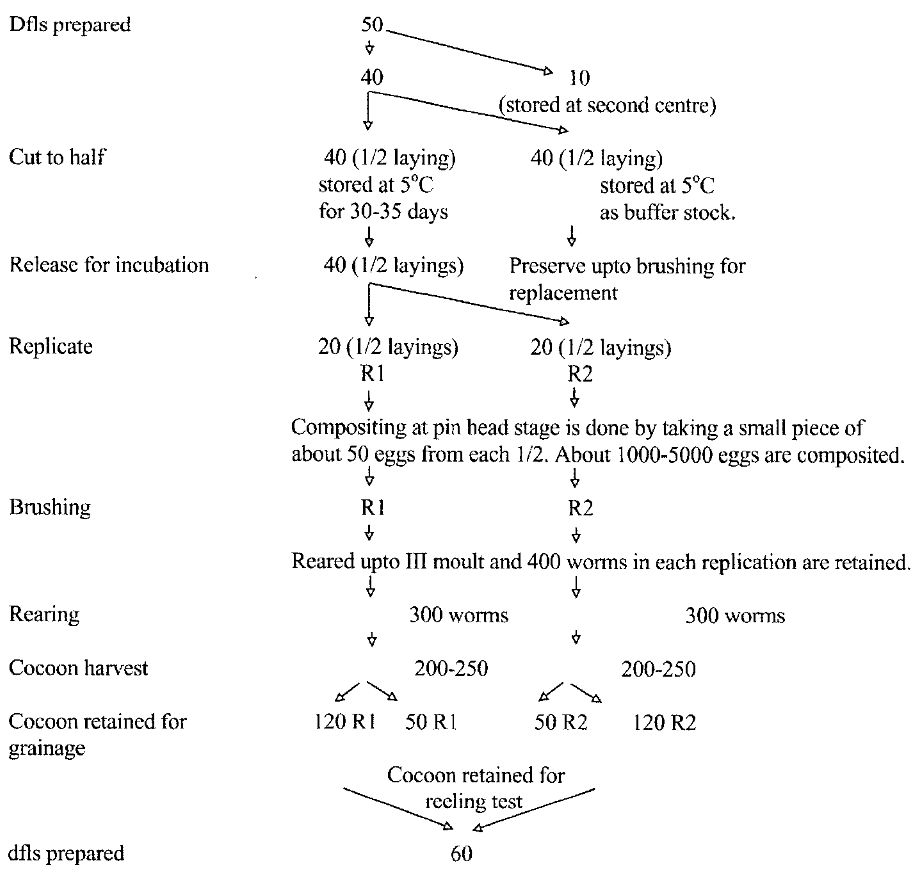

Fig. 1. Plan of multivoltine silkworm stock maintenance.

definite period is highly desirable. Omura (1936) succeeded in artificial insemination in *B. mori*. Kusuda *et al.* (1985), who transplanted frozen thawed ovaries of silkworm into female larvae, recovered the mature eggs from the host and obtained hatched larvae by parthenogenetic activation of the egg. Further, a reliable method is reported for the long-term preservation of ovaries and spermatozoa of the silkworm *B. mori* (Mochida *et al.*, 2003). However, cryopreservation of fertilized eggs has not been successful in silkworm. Few works were undertaken with limited number of accessions to develop a methodology for preserving the eggs of multivoltine silkworm (Govindan and Narayanaswamy, 1986; Yus *et al.*, 1993; Meera verma and Chauhan, 1996). The observation revealed that multivoltine silkworm accessions might be preserved successfully up to 30 days at 5°C. The preliminary studies conducted at this centre (CSGRC) showed that 41 accessions among the available multivoltine silkworm genetic resources could be preserved upto 45 days as they did not show any significant variation for both morphological as well as quantitative traits; and further studies are required to confirm the results, which will reduce conservation cost considerably.

Morphological characterization of multivoltine silkworm genetic resources

The hereditary traits studied in *B. mori* accounts to 211, the second largest number of such traits studied among insects next to that of Drosophila (Yokoyama, 1958). Description of a line or variety based upon a trait, that reflects genetic variation, can be used to measure genetic diversity and can, therefore, be used to monitor and promote efficient conservation and utilization of genetic diversity (Smith and Smith, 1992). The morphological traits are oligogenic in nature inherited under Mendelian genetics and these traits have direct or indirect relation with other quantitative traits. These non-numeric parameters play an indirect basis for selection of parent stocks (initial material). Characterization of morphological features plays a vital role in the identification, accessioning and maintenance of the germplasm. It also provides useful information to the breeders. All the multivoltine silkworm genetic resources have been morphologically characterized and differentiated for their morphological features such as egg, larval, cocoon, pupa and adult stages and details are presented in Table 2. Further, significant and positive correlation was observed between numeric and non-numeric parameters of multivoltine silkworm genetic resources and found that the cocoon colour and shape could be considered as a indirect marker for selection of elite silkworm genetic resources (Kumaresan *et al.*, 2004b).

Table 2. Morphological characterization of 63 multivoltine silkworm accessions available at CSGRC

Sl. no.	Characters	Classification	No. of accessions
	EGG		
1	Egg shape	Ellipsoidal	63
2	Egg colour	Light yellow	48
		Creamy white	5
		White	10
3	Yolk colour	Yellow	47
		Creamy white	12
		Colourless	4
4	Serosa colour	Brown	1
		Olive green	1
		No Serosa colour	61
	LARVA		
1	Colour of neonate	Brown	33
		Yellowish brown	30
2	Larval markings	Plain	40
		Marked	20
		Sex limited	3
3	Body colour	White	8
		Creamy white	51
		Greyish white	4
4	Body built	Slender	10
		Medium stout	42
		Stout	11
5	Nature of Integument	Opaque	7
		Moderately translucent	34
		Translucent	22
6	Haemolymph colour	Yellow	24
		Colourless	39
	COCOON		
1	Cocoon colour	White	13
		Greenish yellow	14
		Creamy white	6
		Yellow	4
		Light greenish yellow	9
		Golden yellow	17
2	Cocoon shape	Elongated oval	24
		Oval	20
		Dumb bell	3
		Spindle	16

Table 2. Continued

Sl. no.	Characters	Classification	No. of accessions
3	Cocoon texture	Fine to Medium	7
		Medium	44
		Fine	12
	PUPA		
1	Pupa colour	Brown	19
		Yellowish brown	44
2	Pupa shape	Ellipsoidal	63
	ADULT		
1	Body colour	Dull white	9♂ 28♀
		Creamy white	18♂ 34♀
		Dirty white	36♂ 1♀
2	Antenna colour	Brown	54
		Yellowish brown	5
		Black	4

Evaluation of multivoltine silkworm genetic resources

Evaluation of germplasm is an essential pre-requisite for utilization. As the goals of breeding change rapidly, the evaluation needs to be adaptive. Evaluation of genetic resources is the most important aspect of germplasm management, which decides the use of genotypes in various programmes of race improvement. The stock and race difference in various biological characters are considered to be the result of adaptation during long generation (Murakami, 1994). The germplasm stocks could be utilized for direct utilization of local breeds or as a parent material, whereas the international need focus towards germplasm systems that emphasize the use and employment of materials rather than more acquisition and storage (Ramesh babu *et al.*, 2001). The silkworm genetic resources are evaluated for rearing, grainage and raw silk parameters during the favourable seasons following set descriptors. Evaluation also helps in identifying the genotypes with specific larval, cocoon and raw silk characters; and also those with resistance to diseases, tolerance to biotic and abiotic stress conditions etc. The identified genotypes can be used as donor parents in breeding programmes. Genetic divergence among the selected geographical races and evolved strains of multivoltine silkworm genetic resources on the basis of economic traits and cocoon characters were studied (Kumaresan *et al.*, 2002 and 2003a). All the 63 multivoltine silkworm genetic resources have been evaluated for economically important characters such as rearing, reeling and yarn quality parameters. The data for the mean performance of all the important economic parameters of 63 multivoltine accessions are presented in Table 3.

Genetic variation in quantitative traits of multivoltine silkworm germplasm

The existence of high genetic variability in economic characters is obviously a resource for breeding (Frankel and Brown, 1983). Based on genetic variation for economic traits and selection indices some best parental silkworm strains of multivoltine were identified (Kumaresan *et al.*, 2000). Variation on genetic parameters *viz.*, phenotypic coefficient of variation (PCV %), genotypic coefficient of variation (GCV %) and heritability (h^2 in broad sense) were estimated for 27 economic traits that includes 11 growth and 16 raw silk traits. Analysis of variance revealed significant variability ($P < 0.01$) for all the traits among the multivoltine silkworm germplasm. In general, PCV % was found to be higher than the GCV % in all the selected traits. The h^2 showed ranges from 4.55% (pupation rate %) to 95.99% (Boil-off loss %). The PCV %, GCV % and h^2 have showed higher in Non-broken filament length (31.79%, 30.93% and 94.66%) followed by Evenness % (58.76%, 45.54% and 60.07%). The shell weight has showed higher PCV (17.21%), GCV (12.93%) and h^2 (56.47%) when compared to other cocoon traits. The results suggested that selection based on above characters would be highly effective for silk improvement (Table 4).

Pre-breeding strategies for the utilization of silkworm germplasm

Pre-breeding may be necessary for the evaluation of some useful characters, it is the early phase of any breeding programme utilizing exotic germplasm (Frankel, 1989). The pre-breeding deals with the extraction of desirable traits; such as disease tolerance or thermo-tolerance, from unadapted unimproved or wild donors and their transfer into high yielding, adapted and improved backgrounds. The derived lines can then be used by breeders in crosses with other elite adapted lines, with knowledge that they have a high probability of being able to select from such crosses commercially acceptable cultivars carrying the desired traits. Some elite multivoltine silkworm accessions were tested for their heterotic effect with popular bivoltine race (Kumaresan *et al.*, 2003b). Pre-breeding is often a key factor in the utilization of wild germplasm (Marshall, 1989). Thus it is imperative to enrich the germplasm collections with wild relatives and exotic gene resources for better utilization of silkworm germplasm in the breeding programmes.

Promising multivoltine silkworm accessions for future utilization

In general, silkworm germplasm is utilized for quality

Table 3. Mean performance of multivoltine silkworm genetic resources for economic characters

Sl. no.	Race	Fecundity	Hatching %	Total larval duration (Days : Hrs)	Fifth age larval duration (Days : Hrs)	Cocoon yield (kg)/ 10,000 larvae	Pupation rate (%)	Single cocoon weight (g)	Single shell weight (cg)	Shell ratio (%)	Raw silk (%)	Filament length (m)	Denier	Reelability (%)	Neatness (%)
1	C.NICHI	409	75.0	22:05	5:01	7.9	87.8	0.95	10.9	11.6	5.5	277	1.71	70.9	49.5
2	RONG DAIZO	466	83.3	24:12	7:11	12.1	77.5	1.37	19.4	14.3	6.5	266	2.10	71.0	72.0
3	GUANGNONG PLAIN	450	91.8	22:23	5:11	8.9	85.8	1.09	16.3	15.1	6.8	365	1.33	64.8	75.5
4	RAJ	436	88.0	24:19	7:03	8.3	81.8	1.07	16.5	15.5	6.9	502	1.25	67.7	92.0
5	GNM	418	86.7	23:11	6:08	10.1	83.9	1.12	18.6	16.6	12.7	519	2.83	65.6	26.0
6	NISTID(Y)	456	92.8	23:20	6:07	8.6	81.8	1.17	16.6	14.4	8.6	368	2.85	44.9	65.5
7	NISTID(W)	411	94.7	24:05	6:09	9.6	90.0	1.04	13.7	13.4	7.9	427	2.16	57.5	81.5
8	NK_4	377	90.3	24:08	6:18	9.6	91.0	1.22	17.9	14.9	7.7	413	1.96	71.8	83.0
9	CAMBODG	349	86.8	23:21	6:13	9.6	85.2	1.19	18.9	16.3	10.4	449	2.35	72.2	81.5
10	DAIZO	399	84.6	23:21	6:20	7.3	76.2	0.93	13.0	14.2	8.1	311	2.10	50.1	84.0
11	PURE MYSORE	439	87.5	27:12	8:01	8.7	78.9	1.04	15.4	15.0	8.4	307	2.44	75.9	71.5
12	SARUPAT	437	83.5	23:11	6:05	9.5	85.1	1.13	17.6	15.9	7.9	351	2.21	81.6	85.0
13	MORIA	437	91.8	24:06	6:17	10.7	87.7	1.13	18.1	16.4	9.3	419	2.22	83.7	89.0
14	TAMILNADU WHITE	446	88.5	24:02	6:13	9.6	83.9	1.16	19.6	17.3	9.7	429	2.32	79.0	93.0
15	HOSA MYSORE	508	84.0	23:16	6:08	10.8	87.9	1.32	26.7	21.0	9.7	515	2.17	79.5	87.0
16	MYSORE PRINCESS	507	86.6	23:20	6:10	10.6	89.8	1.22	19.8	16.4	5.6	326	2.01	77.3	88.5
17	KOLAR GOLD	490	88.1	23:21	6:09	11.4	85.8	1.27	20.5	16.4	7.7	548	1.64	73.6	93.0
18	KOLLEGAL JAWAN	525	92.7	23:12	6:03	11.2	90.2	1.32	20.7	15.9	7.9	699	1.64	82.4	85.0
19	MY_1	461	88.7	24:08	6:14	10.9	88.7	1.33	19.0	14.6	5.4	544	1.14	74.3	68.0
20	P_2D_1	498	91.7	24:03	6:14	10.9	88.1	1.28	20.5	16.1	8.0	475	1.95	72.8	90.5
21	OS_{616}	438	91.1	23:21	6:10	9.2	85.7	1.15	16.7	14.6	7.6	621	1.35	70.8	89.0
22	G	450	89.9	23:12	6:04	10.4	90.0	1.17	19.0	16.4	7.6	598	1.28	76.9	89.0
23	NISTARI	407	79.6	23:10	6:09	8.7	81.5	1.06	15.1	14.4	7.3	399	1.91	76.0	83.5
24	NISTARI(M)	393	91.7	23:13	6:10	9.3	85.7	0.97	14.6	15.1	7.4	287	2.70	73.7	77.5
25	NISTARI(P)	419	91.6	23:18	6:11	9.0	85.6	1.05	14.5	14.1	8.1	432	1.98	73.1	87.0
26	ZPN(SL)	341	85.5	24:04	6:19	8.0	80.3	1.01	15.0	15.0	6.6	301	2.09	79.6	63.5
27	CB_5	390	89.1	24:08	6:21	9.4	87.5	1.10	16.8	15.4	10.0	563	2.16	85.6	71.0
28	KW_2	391	88.6	24:06	6:19	9.1	90.9	1.08	15.9	15.0	8.8	393	2.21	66.5	82.0
29	M_2	441	93.0	23:21	6:20	10.4	89.4	1.08	16.2	15.3	8.9	519	2.61	48.6	84.0
30	A_{23}	425	92.4	24:01	6:08	9.3	80.7	1.16	18.2	15.9	8.3	442	2.40	76.7	74.0
31	A_{25}	476	91.6	24:08	6:17	10.1	84.6	1.23	18.9	15.4	8.0	520	2.01	67.2	84.0
32	OVAL	435	91.7	24:08	6:22	9.3	85.6	1.17	18.0	15.7	9.5	528	2.57	66.9	79.5
33	O	438	93.1	24:04	6:19	10.2	91.8	1.24	20.4	16.6	11.2	526	2.62	78.0	76.5

Table 3. Continued

Sl. no. Race	Fecun-dity	Hatching %	Total larval duration (Days : Hrs)	Fifth age larval duration (Days : Hrs)	Cocoon yield (kg)/ 10,000 larvae	Pupation rate (%)	Single cocoon weight (g)	Single shell weight (cg)	Shell ratio (%)	Raw silk (%)	Filament length (m)	Denier	Reela-bility (%)	Neatness (%)
34 M_{83}(C)	388	88.3	23:19	6:11	8.8	89.8	1.07	17.3	16.4	9.5	402	2.49	74.7	63.0
35 B	454	93.2	23:21	6:13	9.6	92.9	1.10	18.1	16.5	8.7	563	1.79	78.0	85.0
36 A_{14}DY	444	92.1	23:13	6:16	10.5	90.3	1.20	18.3	15.4	9.7	470	2.50	76.6	68.0
37 A_4E	477	83.4	24:06	6:15	10.8	90.2	1.30	22.4	17.3	7.1	570	2.63	50.1	81.5
38 PA_{12}	486	92.9	25:01	7:01	10.1	77.9	1.26	21.0	16.7	11.0	517	2.78	54.1	82.5
39 AP_{12}	474	90.7	24:14	6:22	11.1	79.4	1.38	23.3	16.9	10.0	610	2.70	45.8	82.0
40 A_{13}	503	91.4	23:21	6:15	11.1	88.4	1.40	21.0	15.3	5.1	344	2.07	50.0	80.0
41 PMX	484	92.9	23:20	6:08	10.9	88.5	1.30	21.1	16.3	6.2	281	2.74	66.0	63.0
42 PMS_2	450	92.0	23:18	6:10	10.8	89.3	1.11	17.3	15.8	8.2	378	2.80	73.8	63.0
43 MU_1	486	91.4	23:21	6:07	10.3	88.6	1.33	20.5	15.7	7.8	433	2.15	70.6	90.0
44 MU_{11}	466	90.9	24:00	6:11	10.1	86.7	1.26	20.3	16.3	8.3	413	2.30	78.6	75.0
45 WAI_1	483	92.3	23:18	6:04	9.4	74.5	1.29	18.4	14.5	8.2	440	2.50	64.5	67.0
46 WAI_4	383	91.1	23:08	5:20	8.2	73.8	1.06	15.8	15.0	10.3	501	2.44	78.1	80.0
47 MY_{23}	469	92.5	23:12	6:06	10.4	87.9	1.28	18.8	14.7	8.3	350	2.30	62.3	88.5
48 MW_{13}	476	91.8	24:02	6:12	12.3	88.9	1.33	20.7	15.8	11.6	594	2.30	73.2	83.5
49 MHMP(W)	463	89.0	24:04	6:11	10.0	80.3	1.19	17.6	14.7	9.0	402	2.51	53.8	80.0
50 MHMP(Y)	530	93.5	24:09	6:21	10.8	89.2	1.43	22.9	16.1	9.0	495	2.40	65.5	55.0
51 P_4D_3	515	85.1	24:09	7:00	10.2	86.8	1.27	21.1	16.9	6.8	354	2.25	40.3	82.0
52 LMP	371	90.1	23:15	6:08	9.7	89.3	1.12	16.8	15.2	9.7	402	2.34	84.6	81.5
53 DMR	379	91.0	23:21	6:13	9.5	89.7	1.17	18.3	15.5	10.3	432	2.59	58.9	67.0
54 LMO	439	90.2	23:18	6:08	11.0	87.7	1.21	18.3	15.2	9.9	567	1.84	76.4	86.0
55 MY_1(SL)	491	88.6	24:11	7:07	9.9	86.0	1.25	20.6	16.6	8.4	423	2.24	55.5	58.0
56 PM(SL)	485	91.8	23:22	6:12	10.1	82.7	1.30	19.4	15.2	9.0	425	2.80	61.3	85.0
57 BL_{23}	495	93.1	23:20	6:20	12.1	80.9	1.38	23.0	17.0	5.9	308	2.55	60.0	82.0
58 BL_{24}	514	82.0	23:23	6:16	12.8	81.9	1.39	22.0	15.7	5.8	275	2.91	62.2	60.5
59 MU_{303}	543	90.1	23:20	6:12	11.8	81.6	1.40	20.0	14.8	7.6	327	2.91	65.7	76.5
60 MU_{520}	429	92.4	23:07	6:02	11.6	78.8	1.36	21.0	15.5	8.4	419	2.57	81.7	79.0
61 MU_{10}	309	82.8	23:21	6:11	8.5	67.5	1.30	21.0	16.5	7.3	402	2.28	72.5	78.5
62 TW × SK_6 × SK_1	397	93.4	23:09	5:18	8.7	70.6	1.06	13.0	12.1	5.3	262	2.20	86.5	56.5
63 SK_6 × SK_1 × TW	523	84.0	23:14	5:00	9.7	80.4	1.05	12.0	11.3	5.9	246	2.35	86.1	56.0

Table 4. Genetic variation in quantitative traits of multivoltine silkworm germplasm

Sl. no	Variables	Mean	SE	CV %	PCV %	GCV %	Heritability (broad sense)	F-Value**
1	Fecundity	447	3.08	18.15	17.65	8.08	29.97	4.18
2	Hatching %	89.6	0.29	8.82	8.69	2.42	7.77	2.01
3	Wt.of 10 matured larvae (g)	25.6	0.17	17.37	15.91	9.53	35.93	7.73
4	Total larval duration (Days:Hrs.)	23:14	1.65	7.69	5.28	2.48	22.03	4.39
5	V age larval duration (Days:Hrs.)	6:06	0.75	13.21	11.52	6.62	33.04	6.92
6	Cocoon yield (No.)/10000 larvae	8567	34.12	10.51	9.69	2.3	5.64	1.72
7	Cocoon yield (Kg)/10000 larvae	10.4	0.07	19.16	17.62	9.19	27.17	5.48
8	Single cocoon wt.(g)	1.25	0.01	14.89	12.99	8.85	46.45	11.44
9	Single shell wt.(cg)	18.6	0.001	18.23	17.21	12.93	56.47	16.58
10	Shell ratio (%)	15.0	0.05	8.51	8.05	4.92	37.31	8.14
11	Pupation rate (%)	83.8	0.42	13.09	11.78	2.51	4.55	1.57
12	Filament length (m)	441	9.66	23.6	23.71	22.72	91.85	23.53
13	Non-broken filament length (m)	306	8.99	31.65	31.79	30.93	94.66	36.47
14	Denier (d)	2.2	0.04	19.86	19.91	18.89	90.03	19.06
15	Reelability (%)	68.4	1.05	16.49	16.55	15.68	89.73	18.48
16	Renditta (Kg)	12.5	0.24	20.97	21.05	20.45	94.36	34.49
17	Raw silk recovery (%)	56.4	1.07	20.45	20.52	19.18	87.33	14.79
18	Waste (%)	28.0	0.89	34.24	34.39	32.14	87.33	14.79
19	Raw silk (%)	8.3	0.15	19.7	19.78	19.11	93.31	28.9
20	Neatness (%)	77.4	1.33	18.53	18.58	12.73	46.96	2.77
21	Boil-off-loss (%)	21.7	0.27	13.41	13.45	13.18	95.99	48.99
22	Cleanness (%)	78.6	1.67	22.93	22.79	17.16	56.66	3.61
23	Evenness V-1 (count)	37.8	2.06	58.76	58.76	45.54	60.07	4.01
24	Tenacity (g/d)	3.3	0.29	9.39	9.43	8.19	75.61	7.2
25	Elongation (%)	14.9	0.23	16.38	16.41	11.84	52.05	3.17
26	Cohesion (strokes)	46.4	2.32	53.95	54.18	38.89	51.53	3.13
27	Low- neatness (%)	70.3	1.54	23.62	23.69	16.69	49.69	2.98

**Significance at $p < 0.01$.

improvement and commercial exploitation. Screening out the promising pure breeds from the germplasm stocks is an important duty of the curator of gene bank maintenance. Ten best accessions based on better performance over three years (1997 – 2000) have been screened out under Hosur climatic condition for the major economically important 11 rearing and 14 raw silk quality parameters. These promising accessions are observed to be better than the popular commercial breeds like Pure Mysore, Nistari and multi × bi hybrids. These accessions are Kollegal Jawan, Kolar Gold, MHMP (Y), AP_{12}, MW_{13}, Hosa Mysore, LMO, P_4D_3, A_{13} and CB_5 (Table 5). Further, some multivoltine strains were identified for high temperature (36°C) tolerance (Koundinya *et al.*, 2003). These selected accessions could be utilized either for the breed improvement programme or as one of the maternal parents in the crossbreed development for commercial exploitation.

Utilization of multivoltine silkworm genetic resources

Conservation must be accompanied by effective utilization of germplasm on sustainable basis so as to enhance the usefulness of large collections, which will justify long-term investments on conservation of gene pool (Thangavelu and Sinha, 2002). On the other hand, increasing utilization indirectly strengthens the conservation as more and better use of genetic resources provides an incentive for their effective conservation. Thus, there is an urgent need for increased utilization of silkworm germplasm resources both for direct commercial exploitation and indirectly for evolution of high yielding breeds with improved quality. A correct selection of donor parents shall depend on a correct understanding of the germplasm

Table 5. Best ten multivoltine silkworm accessions for various economic traits and yarn quality parameters

Race name	Parameters	No.of rearing/reeling parameters	No.of parameters	Race name	Parameters	No.of rearing/reeling para-meter	No.of parameters
Kollegal Jawan	Fecundity, Weight of 10 grown larvae, Total larval duration, Fifth age larval duration, Single shell weight, Cocoon yield/10,000 larvae by weight, Filament length, Denier, Non-broken filament length, Reelability%, Boil-off loss, Evenness V-II, Tenacity .	6/7	13	Hosa Mysore	Fecundity, weight of 10 grown larvae, Single cocoon weight, single shell weight, Shell ratio, Cocoon yield/10,000 larvae by weight, Non-broken filament length, Reelability%, Tenacity	6/3	9
Kolar Gold	Fecundity, weight of 10 grown larvae, Fifth age larval duration, single shell weight, Shell ratio, Cocoon yield/10,000 larvae by weight, Filament length, Denier, Non-broken filament length Neatness, Boil-off loss, Tenacity, Cohesiveness	6/7	13	LMO	Cocoon yield/10,000 larvae by number, Pupation rate, Filament length, Denier, Renditta, Raw silk%, Cleanliness, Tenacity	2/6	8
MHMP(Y)	Fecundity, Weight of 10 grown larvae, Single cocoon weight, Single shell weight, Cocoon yield/10,000 larvae by number, Cocoon yield/10,000 larvae by weight, Pupation rate, Boil-off loss, Cleanness, Tenacity	7/3	10	P_4D_3	Hatching%, Single cocoon weight, Single shell weight, Shell ratio, Boil-off loss, Cleanliness, Evenness-V-II, Cohesiveness	4/4	8
AP_{12}	Fecundity, Weight of 10 grown larvae, Single cocoon weight, Single shell weight, Shell ratio, Cocoon yield/10,000 larvae by weight, Filament length, Renditta, Raw silk%, Evenness V-II	6/4	10	A_{13}	Fecundity, Hatching%, Weight of 10 grown larvae, single cocoon weight, Single shell weight, Cocoon yield/10,000 larvae by weight, Tenacity, Elongation	6/2	8
MW_{13}	Weight of 10 grown larvae, Fifth age larval duration, Single cocoon weight, Filament length, Non-broken filament length, Renditta, Raw silk%, Boil-off loss, Evenness- V II	3/6	9	CB_5	Cocoon yield/10,000 larvae by number, Filament length, Non-broken filament length, Reelability%, Renditta, Raw silk%, Cleanliness, Cohesiveness	1/7	8

material as well as gene pool from which the suitable donors have to be selected.

An effective utilization of genetic resources depends on the following factors:

1. Rapid characterisation and evaluation of germplasm collection.
2. Development and use of efficient screening technique.
3. Classification of germplasm materials based on genetic divergence and geographical distances.
4. Establishment of regional centre (agro-climatic regions) for germplasm conservation and evaluation under natural conditions, to serve as backup and also for correct characterization.
5. Enrichment of germplasm with wild and exotic collections.
6. Evaluation of genetic resources in varied agro-climatic regions, seasons and hot spot evaluation for biotic and abiotic factors.
7. Pre-breeding activities with stress on base broadening linked with main silkworm breeding programme of the institutes.
8. Promotion of non-sericulture use of silkworm *viz.,* to study various biological processes using silkworm as a bioreactor.
9. Strengthening facility for computerized gene banks for data recording, processing and retrieval.
10. Un-adapted exotic collections can be pre-bred with indigenous well-adapted low productive breeds to make genetic complexes for extraction of valuable genes through hybridization/selection breeding. Base broadening can be done to create new variability, which could attract the breeders for effective utilization (Kumaresan *et al.,* 2004a).

Since we do not have sufficient useful multivoltine breeds with specific characters like longer filament length, thin denier, thicker denier, disease tolerance etc., there is a need to develop such breeds to act as resource material for future breeding programme.

Conclusion

In Indian sericulture, the major portion of sericulture depends on multivoltine races either as pure breeds or cross breeds (multi × bivoltine) for silk production, since the climatic conditions are not ideally suitable for promotion of bivoltine sericulture. Therefore, there is an urgent need to collect more number of existing multivoltine breeds from within as well as outside the country to enrich the genetic resources, which will contribute to promote Indian silk industry through productive and qualitatively superior breeds/hybrids.

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