

Effect of Extended Egg Preservation Schedule in Conservation of Mutant Silkworm (*Bombyx mori* L.) Genetic Stocks in Gene Bank

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Studies on extended egg preservation schedule from 120 days to 180 days was taken up with 20 germplasm accessions of mutant silkworm genetic stocks of *Bombyx mori* L. Statistical analyses of the data collected over three trials revealed no significant changes both in the qualitative and quantitative traits of the genetic stocks between treatment (6 months egg preservation) and control (4 months egg preservation), except for fifth instar larval duration in TMS-61, TMS-62, TMS-64, TMS-31 and TMS-34 shell weight in TMS-62, TMS-64 and TMS-66. Thus, the results indicate that extended schedule of 6 months egg preservation can safely be adopted, which will reduce the cost of conservation and minimize the genetic erosion owing to reduced crop cycle.

Key words: *Bombyx mori*, Silkworm, Mutants, Genetic stocks, Cold preservation

Introduction

Several workers have undertaken studies on preservation of eggs of multivoltine silkworm (Higashi, 1971; Datta *et al.*, 1972; Govindan and Narayanaswamy, 1986; Narayanasamy and Govindan, 1987; Tayade *et al.*, 1987; Manjula and Hurkadli, 1993; Yu *et al.*, 1993; Meera Verma and Chauhan, 1996). In the case of bivoltine mutant genetic stocks, 4 months egg preservation schedule is followed, since mutant genetic stocks failed to tolerate 10 months cold storage schedule as practiced in the case of bivoltine genetic resources. In order to reduce the cost of

conservation and labour, an attempt was made to develop and standardize 6 months egg preservation schedule for mutant genetic stocks of *B. mori* with an objective to minimize the crop cycle per year as successfully done in case of multivoltine silkworm germplasm (Kumaresan *et al.*, 2004).

Preliminary evaluation trials with 4 months, 6 months and 8 months conducted for hatching indicated encouraging results in 4 months and 6 months preservation whereas 8 months results showed considerable reduction in egg hatching in few accessions. Accordingly, based on the results of preliminary studies, detailed experimentation was undertaken with 6 months preservation schedule to study its effect on growth and reproductive traits. The outcome of the study are reduction in the cost of conservation, reduction in silkworm crop cycles from 3 to 2 year, minimized exposure to diseases and genetic erosion.

Materials and Methods

Present investigation was undertaken with 20 mutant genetic stocks received from Central Sericultural Research and Training Institute, Mysore presently being maintained at the center. The donor institute was maintaining the mutant stocks under four months preservation schedule. Twenty bivoltine mutant genetic stocks of Japanese origin constituted the materials for the present study during the year 2004 – 2005 (Table 1). Eggs preserved for 4 months schedule was kept as control, while eggs preserved for 6 months schedule was considered as treatment. After the completion of experimental preservation period, the eggs were incubated at $25 \pm 1^\circ\text{C}$ and $85 \pm 2\%$ RH. Standard silkworm rearing technique was adopted throughout the rearing period using composited layings (Thangavelu *et al.*, 2000), following completely randomised block design (CRBD) with two replications. From neonate to third moult all the larvae of composited

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Table 1. List of mutant silkworm genetic stock of *Bombyx mori* considered for egg preservation for 4 and 6 months preservation schedule

Sl. no.	Name	Trait(s) & linkage group
1	TMS-2	striped larval body markings (pS, 2:0.0).
2	TMS-12	zebra body markings (Ze, 3:20.8).
3	TMS-14	red haemolymph (rb, 21:0.0).
4	TMS-17	lemon larval body colour (lem, 3:0.0).
5	TMS-31	tubby larval body shape (tub, 23:6.9).
6	TMS-32	stony larval body shape (st, 8:0.0).
7	TMS-33	blind larval body markings (bl, 15:0.0), cheek & tail spots (cts, 18:4.6).
8	TMS-34	egg colour (pe, 5:0.0) and brown head & tail spots (bts, 17:30.1).
9	TMS-35	white egg colour (w-2, 10:16.1) chocolate neonate colour (ch, 13:9.6), sooty larval body colour (so, 26:0.0) and melanism larval body markings (mln, 18:41.5).
10	TMS-38	ursa larval body colour (U-2, 14:40.5), extra-leg body shape (E, 6:0.0) and wild wing spot (Ws, 17:14.7).
11	TMS-61	brownish red egg colour (b-2, 6:8.0), red egg (re, 5:31.7), blind larval body mark (bl, 15:0.0) and lustrous eyes in moths (lu, 16:0.0).
12	TMS-62	ellipsoidal egg shape (elp, 18:16.1) and melanism in head & anal plates of larva (mln, 18:41.5).
13	TMS-64	chocolate neonate colour (la, 9:22.1), lemon coloured larval body (lem, 3:0.0) and Chinese translucent skin (oc, 5:40.8).
14	TMS-65	narrow-brest larval body shape (nb, 19:31.2), moricaud larval marking (pM, 2:0.0) and r-translucent integument (or, 22:8.9).
15	TMS-66	zebra faded larval marking (ZeF, 3:20.8) and wild wing spot on wings (Ws, 17:14.7).
16	TMS-67	yellow haemolymph colour (Y, 2:25.6) and pink and flesh coloured cocoons (Pk, 2:? ; F, 6:13.6) colour and shape.
17	TMS-69	multi-lunar larval body markings (L, 4:15.3).
18	TMS-75	red egg colour (re, 5:31.7), elongated II & III abdominal segments (e, 1:36.4), quail body markings (q, 7:0.0) and sex-linked translucent integument (os, 1:0.0).
19	TMS-82	knobbed larval body shape (K, 11:23.2).
20	ODT	translucent larval body cuticle.

layings were maintained as such and three hundred fourth instar larvae were maintained in each replication for further data recording.

Evaluation data on important economic parameters *viz.*, fecundity, hatching percentage, weight of 10 grown larvae, total larval duration, fifth age larval duration, cocoon yield (No.) / 10000 larvae and cocoon yield (weight) / 10000 larvae, pupation percentage, single cocoon weight, single shell weight and silk ratio were recorded during the experimentation. Confirmatory morphological characterization for larval, pupa, cocoon and moth stages were carried out based on the passport data. After cocoon assessment, live cocoons were maintained at $25 \pm 1^\circ\text{C}$ and $85 \pm 2\%$ RH. Disease free layings produced from the control and treated batches were preserved for 4 and 6 months preservation following the standardized egg preservation schedules (Table 2) developed by Manjula and Hurkadli (1995) in order to confirm the earlier results. Three trials were conducted during the experimental period. First trial

Table 2. Egg preservation schedule adopted for mutant genetic stocks of *Bombyx mori*

Temperature (°C)	Treatment (6 months)	Control (4 months)
25	20	10
20	3	3
15	3	3
10	3	3
5	147	97
15	3	3
20	1	1
25	Release for incubation	Release for incubation
Total	180 days	120 days

during February to March 2004, second trial during August to September 2004 and third trial during March to

Table 3. Continued

Name Details	Fecundity (no.)	Hatching %		Larval weight (g)		Total larval duration (h)		V instar larval duration (h)		Yield/10,000 larvae (no.)		Yield/10,000 larvae (kg)		Pupation rate (%)		Cocoon weight (g)		Shell weight (g)		Shell ratio			
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TMS-61	4 M	373 ± 26		94.2 ± 0.71		19.7 ± 0.79		538 ± 12		114 ± 5.8		9788 ± 59.8		8.7 ± 0.36		95.1 ± 1.36		0.84 ± 0.06		0.11 ± 0.007		13.4 ± 0.26	
	6 M	367 ± 20.3		93 ± 2.1		17.8 ± 0.44		541 ± 3.6		125 ± 6		9776 ± 30.3		8.6 ± 0.76		93.3 ± 1.2		0.89 ± 0.06		0.11 ± 0.01		12.7 ± 0.5	
TMS-62	4 M	399 ± 18.2		86.1 ± 1.23		23.3 ± 2.63		539 ± 9.9		115 ± 4		9518 ± 192		10.5 ± 0.85		92.8 ± 2.28		1.2 ± 0.05		0.15 ± 0.005		12.7 ± 0.12	
	6 M	484 ± 19.5		83.7 ± 4.16		21.9 ± 0.48		539 ± 5.9		136 ± 2.95		9484 ± 120.7		11.7 ± 0.71		89.07 ± 2.3		1.19 ± 0.048		0.14 ± 0.007		12 ± 0.29	
TMS-64	4 M	424 ± 20.2		91.9 ± 1.76		19.4 ± 1.9		539 ± 11.2		115 ± 4.9		9492 ± 83.3		8.2 ± 0.42		90.7 ± 1.4		0.92 ± 0.04		0.13 ± 0.005		13.9 ± 0.12	
	6 M	412 ± 11.3		85.8 ± 3.78		18.9 ± 0.53		545 ± 4.46		129 ± 5.5		9857 ± 25.79		9.3 ± 0.49		94.3 ± 1.42		0.88 ± 0.063		0.11 ± 0.007		13.2 ± 0.21	
TMS-65	4 M	311 ± 23.6		96.2 ± 0.78		21.1 ± 1.1		539 ± 12.4		115 ± 6.2		9785 ± 40.3		8.9 ± 0.77		91.9 ± 2.02		0.94 ± 0.06		0.11 ± 0.07		11.9 ± 0.39	
	6 M	324 ± 50.6		94.1 ± 1.04		20.4 ± 1.31		547 ± 6.58		123 ± 5.9		9832 ± 49.3		9.8 ± 0.89		89.2 ± 2.28		0.97 ± 0.087		0.11 ± 0.009		11.6 ± 0.33	
TMS-66	4 M	252 ± 8.5		81.8 ± 5.5		22.7 ± 2.4		560 ± 3.8		126 ± 4.4		9811 ± 64.4		10.5 ± 0.67		88.6 ± 4.2		1.03 ± 0.08		0.09 ± 0.009		9.2 ± 0.24	
	6 M	277 ± 5.2		81.4 ± 3.63		21.6 ± 1.15		538 ± 8.04		122 ± 5.9		9850 ± 22.4		11.4 ± 0.97		89.1 ± 1.55		1.16 ± 0.05		0.12 ± 0.011		10.1 ± 0.49	
TMS-67	4 M	280 ± 16.4		91.7 ± 1.8		18.2 ± 0.6		542 ± 15.2		118 ± 5.06		9455 ± 109		8.35 ± 0.63		88.7 ± 1.64		0.87 ± 0.06		0.12 ± 0.007		14.4 ± 0.38	
	6 M	249 ± 15.9		85.2 ± 4.3		19.5 ± 0.65		540 ± 4.4		123 ± 7.67		9722 ± 87.5		8.85 ± 0.56		90.9 ± 2.17		0.88 ± 0.06		0.12 ± 0.07		13.5 ± 0.37	
TMS-69	4 M	428 ± 16.9		91 ± 2.02		27.1 ± 1.79		538 ± 9.7		124 ± 3.4		9555 ± 101		13.3 ± 0.82		92.76 ± 2.17		1.26 ± 0.05		0.18 ± 0.006		14.8 ± 0.27	
	6 M	443 ± 8.3		88.9 ± 1.72		25.1 ± 0.23		531 ± 1.1		128 ± 5.06		9337 ± 154.9		14.15 ± 0.94		93 ± 1.66		1.3 ± 0.11		0.18 ± 0.01		15.17 ± 1.41	
TMS-75	4 M	334 ± 27.8		92.2 ± 1.74		18.7 ± 1.2		544 ± 14.4		118 ± 0.073		9761 ± 39.5		8.32 ± 0.74		93.3 ± 1.79		0.87 ± 0.02		0.11 ± 0.005		13.2 ± 0.31	
	6 M	307 ± 22.5		90.2 ± 2.22		18.02 ± 0.8		540 ± 2.92		128 ± 5.3		9857 ± 31.9		9.75 ± 1.13		93.6 ± 1.44		0.89 ± 0.05		0.11 ± 0.005		12.7 ± 0.38	
TMS-82	4 M	393 ± 20.7		96.1 ± 0.78		26.8 ± 1.81		544 ± 14.4		119 ± 0.422		9847 ± 21.7		11.8 ± 0.84		90.9 ± 3.3		1.37 ± 0.05		0.19 ± 0.008		14.3 ± 0.27	
	6 M	424 ± 32.8		90.1 ± 4.2		27.82 ± 1.41		538 ± 3.6		122 ± 6.96		9894 ± 6.93		13.1 ± 1.04		93.2 ± 1.26		1.3 ± 0.1		0.18 ± 0.02		14.1 ± 0.35	
ODT	4 M	410 ± 5.2		97.1 ± 0.91		29.1 ± 1.04		516 ± 13.8		122 ± 5.7		9818 ± 59.9		11.7 ± 0.79		97.3 ± 0.78		1.2 ± 0.08		0.18 ± 0.013		15.4 ± 0.23	
	6 M	408 ± 13.5		97.9 ± 0.44		27.9 ± 0.44		540 ± 0		132 ± 0		9800 ± 6		10.8 ± 0.1		98.9 ± 0.005		1.06 ± 0.08		0.16 ± 0.007		15.38 ± 0.35	
CD at 5%	4 M	48.13		5.41		3.19		26.46		9.42		226		1.38		5.97		0.12		0.018		0.85	
	6 M	65.6		6.24		2.22		12.97		11.84		190.9		1.59		4.69		0.13		0.019		1.35	

4 M- Control 6 M- Treatment.

April 2005. The data recorded for three generations were compiled and statistically analysed using computer Statistical Packages of SPSS Inc., U.S.A.

Results and Discussion

The mean performance of control (4 months egg preservation schedule) and treatment batches (6 months egg preservation schedule) for eleven important economic parameters are presented in (Table 3). The data were subjected to ANOVA, which revealed highly significant variation among the mutant genetic stocks for all the traits in both the batches except for pupation rate in the control (data not shown). The purpose of the study is to compare the rearing performance of the experimental batch (6 months egg preservation schedule) with that of control (4 months egg preservation schedule). Hence the data recorded for three trials for both the batches were com-

pared by using the student's t-test. The results showed no significant difference for most of the economic parameters studied like hatching percentage, larval weight, cocoon weight and shell ratio between treatment and control (Table 4). However few mutant genetic stocks showed significant variation between treatment and control for some characters. In the case of fecundity TMS-62 showed higher value in treatment (484 eggs/df) than control (399 eggs/df). Total larval duration (hrs) decreased by 19 hrs, 22 hrs and 16 hrs in TMS-38, TMS-66 and TMS-34 respectively in treatment batch compared to control. Fifth age larval duration (hrs) was more in TMS-31, TMS-34, TMS-61, TMS-62 and TMS-64 in treatment batch than control. While in the case of Cocoon yield (no.) / 10000 larvae TMS-64 showed significantly higher value of 9857 under 6 months compared to control (9492). Higher Cocoon yield (weight) / 10000 larvae was recorded in TMS-38 (12.5 kg), TMS-65 (9.8 kg) and TMS-82 (13.1 kg) in treatment than control where it was 11.05 kg, 8.9 kg

Table 4. Comparative performance (t-values) of 4 and 6 months egg preservation for economic parameters of 20 mutant genetic stocks of *Bombyx mori*

Name	Fecundity	Hatching %	Larval weight (g)	Total larval duration (h)	V instar larval duration (h)	Yield/ 10,000 larvae (no.)	Yield/ 10,000 larvae (kg.)	Pupation rate (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio
TMS-2	0.08 ^{NS}	1.59 ^{NS}	-0.971 ^{NS}	-0.205 ^{NS}	-0.53 ^{NS}	0.761 ^{NS}	-0.757 ^{NS}	1.126 ^{NS}	-0.73 ^{NS}	-2.498 ^{NS}	-1.053 ^{NS}
TMS-12	1.072 ^{NS}	0.107 ^{NS}	2.503 ^{NS}	1.898 ^{NS}	-2.39 ^{NS}	0.079 ^{NS}	-1.634 ^{NS}	0.644 ^{NS}	-0.64 ^{NS}	-0.416 ^{NS}	1.196 ^{NS}
TMS-14	0.363 ^{NS}	0.051 ^{NS}	2.029 ^{NS}	0.528 ^{NS}	1.581 ^{NS}	0.972 ^{NS}	-3.527 ^{NS}	0.101 ^{NS}	-0.769 ^{NS}	0.855 ^{NS}	-1.87 ^{NS}
TMS-17	-0.193 ^{NS}	-0.321 ^{NS}	1.658 ^{NS}	0 ^{NS}	0.079 ^{NS}	0.149 ^{NS}	0.064 ^{NS}	0.779 ^{NS}	0.682 ^{NS}	0.62 ^{NS}	-0.017 ^{NS}
TMS-31	1.341 ^{NS}	-1.792 ^{NS}	0.592 ^{NS}	-0.508 ^{NS}	-3.784*	-0.695 ^{NS}	-0.348 ^{NS}	1.289 ^{NS}	-0.747 ^{NS}	0.229 ^{NS}	1.573 ^{NS}
TMS-32	1.002 ^{NS}	2.4 ^{NS}	1.652 ^{NS}	-0.478	-5.138 ^{NS}	-0.769 ^{NS}	-2.533 ^{NS}	-0.887 ^{NS}	1.005 ^{NS}	1.636 ^{NS}	2.107 ^{NS}
TMS-33	1.751 ^{NS}	0.392 ^{NS}	1.017 ^{NS}	0.736 ^{NS}	-1.688 ^{NS}	-0.315 ^{NS}	1.389 ^{NS}	0.758 ^{NS}	-2.488 ^{NS}	-0.982 ^{NS}	2.261 ^{NS}
TMS-34	2.367 ^{NS}	2.216 ^{NS}	0.667 ^{NS}	2.877*	-5.192**	1.001 ^{NS}	-1.726 ^{NS}	-0.637 ^{NS}	-0.27 ^{NS}	-0.515 ^{NS}	-2.086 ^{NS}
TMS-35	1.379 ^{NS}	-0.66 ^{NS}	-0.836 ^{NS}	1.712 ^{NS}	0.96 ^{NS}	-0.123 ^{NS}	0.565 ^{NS}	2.051 ^{NS}	-1.616 ^{NS}	-1.598 ^{NS}	0.195 ^{NS}
TMS-38	0.171 ^{NS}	-0.784 ^{NS}	2.294 ^{NS}	5.129**	0.157 ^{NS}	0.602 ^{NS}	-5.4 ^{NS}	-0.02 ^{NS}	-0.467 ^{NS}	-0.684 ^{NS}	0.246 ^{NS}
TMS-61	0.199 ^{NS}	0.495 ^{NS}	2.146 ^{NS}	-0.34 ^{NS}	-3.146*	0.176 ^{NS}	0.15 ^{NS}	1.024 ^{NS}	-2.398 ^{NS}	-0.156 ^{NS}	1.281 ^{NS}
TMS-62	-3.204**	0.708 ^{NS}	0.527 ^{NS}	0 ^{NS}	-19.124**	0.147 ^{NS}	-1.127 ^{NS}	1.778 ^{NS}	0.075 ^{NS}	2.931*	2.212 ^{NS}
TMS-64	0.504 ^{NS}	1.473 ^{NS}	0.313 ^{NS}	-0.834 ^{NS}	-19.17**	-4.182*	-1.675 ^{NS}	-7.091**	1.25 ^{NS}	3.284*	2.959 ^{NS}
TMS-65	-0.251 ^{NS}	1.567 ^{NS}	1.075 ^{NS}	-1.002 ^{NS}	-2.485 ^{NS}	-0.733 ^{NS}	-3.418*	0.91 ^{NS}	-0.52 ^{NS}	-0.035 ^{NS}	0.621 ^{NS}
TMS-66	-2.514 ^{NS}	0.06 ^{NS}	1.559 ^{NS}	2.39*	0.451 ^{NS}	-0.57 ^{NS}	-1.623 ^{NS}	-1.22 ^{NS}	-1.248 ^{NS}	-3.387*	-1.53 ^{NS}
TMS-67	1.35 ^{NS}	1.395 ^{NS}	-1.51 ^{NS}	-1.93 ^{NS}	-1.233 ^{NS}	-1.9 ^{NS}	-2.053 ^{NS}	1.395 ^{NS}	-0.208 ^{NS}	0.461	1.59 ^{NS}
TMS-69	-0.848 ^{NS}	0.782 ^{NS}	1.082 ^{NS}	0.749 ^{NS}	-2.193 ^{NS}	1.173 ^{NS}	-2.049 ^{NS}	-0.12 ^{NS}	-0.713 ^{NS}	-0.094 ^{NS}	-0.191 ^{NS}
TMS-75	0.742 ^{NS}	0.726 ^{NS}	1.457 ^{NS}	0.345 ^{NS}	-2.193 ^{NS}	-1.874 ^{NS}	-1.062 ^{NS}	-0.152 ^{NS}	-0.357 ^{NS}	0.767 ^{NS}	1.093 ^{NS}
TMS-82	-0.785 ^{NS}	1.555 ^{NS}	-1.204 ^{NS}	0.495 ^{NS}	-0.405 ^{NS}	-2.074 ^{NS}	-3.395*	-1.031 ^{NS}	1.431 ^{NS}	0.636 ^{NS}	0.402 ^{NS}
ODT	0.198 ^{NS}	-0.578 ^{NS}	0.745 ^{NS}	-1.155 ^{NS}	-1.555 ^{NS}	-0.206 ^{NS}	0.778 ^{NS}	-1.4 ^{NS} 02	-1.062 ^{NS}	1.08 ^{NS}	0.131 ^{NS}

and 11.8 kg respectively. Higher pupation rate was recorded in treatment batch of TMS-64 (94.3%) and in control it was 90.7%. Regarding fifth age larval duration, TMS-31, TMS-34, TMS-61, TMS-62 and TMS-64 showed negatively significant 't' value, in other words fifth age larval duration is more under treatment batch (16 – 22 hrs) which may be due to extended preservation in low temperature. The important characters like cocoon yield (number) / 10000 larvae (TMS-64), Cocoon yield (weight) / 10000 larvae (TMS-38, TMS-65, TMS-82), pupation rate (TMS-64), shell weight (TMS-62 and TMS-64), treated batches showed significantly higher values.

Observation on highly heritable morphological traits *viz.*, larval markings, cocoon colour, cocoon shape and moth characteristics, moth emergence pattern and oviposition pattern revealed no significant change between the treatment and control batches throughout the experimental period. This corroborates the results obtained with multivoltine silkworm genetic resources, where extended egg preservation for 45 days against 30 days, did not show significant changes both in the qualitative and quantitative traits (Kumaresan *et al.*, 2004). And also, utilisation of mutant silkworm is widely adopted by silkworm breeders. Some of the mutations for economical characters are directly utilized in silkworm breeding, the polyphagous mutation had been utilized to breed silkworm varieties adaptable to artificial diet to produce fine silk. The non-glutinous mutation was used to produce natural loose eggs. Some of the morphological characters have multiple functions, which also have positive effect on the economic characters. Therefore, it is possible to use mutant genetic stocks directly in silkworm breeding for evolving new races. Significant variation observed between treatment and control for characters like larval duration cocoon yield (no.) / 10,000 larvae, cocoon yield (weight) / 10,000 larvae are mainly due to the influence of the environment. The variation observed for fecundity, shell weight, larval duration and pupation rate in few accessions over control batch did not show considerable deviation, which predisposes the fact that these accessions can safely be maintained in the gene bank without any adverse change in their genetic traits. Further, conservation following 6 months egg preservation helps to minimize the crop cycle cost and labour involved in rearing and grainage. This will help to reduce genetic depression / genetic erosion and minimize the exposure to biotic and abiotic stress factors and it facilitates rearing of mutants in favorable seasons.

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