

Studies on Long-term Preservation of Eggs of Indian Tropical Multivoltine Silkworm (*Bombyx mori* L.) Genetic Resources

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The silkworm rearing and growth parameters of 63 multivoltine silkworm accessions under extended period of egg preservation at 5°C from 30 days to 45 days were studied. The results indicate that, nine accessions did not respond to extended period of egg preservation at low temperature and the remaining 54 accessions responded to the treatment and three rearings were conducted for comparison with the control; to estimate the effect of prolonged egg preservation at low temperature. The non-parametric tests statistics (Wilcoxon tests) was adopted for comparing the mean performance of treated batches (45 days) over the control (30 days). Highly significant variability was found among the accessions for all the parameters under study. The genetically controlled morphological characters were not altered in the treated batches, which were found to be on par with that of control. However, the total larval duration varied significantly over the control in 51 accessions. Similarly, the fifth age larval duration of 27 accessions showed decreasing trend compared to control. Altogether 41 accessions were found to be tolerant to long-term cold preservation upto 45 days, without showing any significant variation for morphological as well as essential quantitative traits. These accessions may be recommended for long-term egg preservation schedule up to 45 days, which will reduce the cost of conservation of these silkworm germplasm.

Key words: Silkworm, Germplasm, Multivoltine, Cold preservation

Introduction

In general, maintenance of silkworm (*Bombyx mori* L.) genetic resources is quite different from that of agricultural gene bank. The multivoltine silkworm accessions have to be reared for 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. 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. However, development of long-term conservation is obligatory as the accessions are escalating with the introduction of new genetic resources over the years.

Several workers have undertaken studies on preservation of eggs of multivoltine silkworm with very limited number of accessions (Higashi, 1971; Datta *et al.*, 1972; Govindan and Narayanaswamy, 1986; Narayanaswamy and Govindan, 1987; Tayade *et al.*, 1987; Manjula and Hurkadli, 1993; Yu *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. Central Sericultural Germplasm Resource Centre (CSGRC), Hosur (12.45°N, 77.51°E, 942 m above MSL) has been maintaining 63 multivoltine silkworm genetic resources following five crops per year by adopting 30 days cold preservation of eggs at 5°C after 30 hrs of oviposition at room temperature (25°C).

Present investigation has been under taken to study the effect of 45 days egg preservation at 5°C considering rearing and growth parameters of 63 multivoltine silkworm

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accessions with the objectives: i) to ascertain the differential response of multivoltine accessions to long-term egg preservation, ii) to reduce the crop cycle and avoid the genetic erosion and iii) to curtail the cost of conservation.

Materials and Methods

The multivoltine silkworm accessions (53 indigenous and 10 exotic) of different origin constituted the materials for the present study. The eggs of all the accessions were preserved in two batches at 5°C after 30 hrs of oviposition at room temperature 25°C. The eggs of one set were preserved at 5°C for 30 days that was considered as control and another set was preserved up to 45 days as treatment batch. The eggs were released following intermediate temperature of 15°C (2–3 hrs) and incubated at 24–25°C and 80% RH. The standard silkworm rearing technique (Krishnaswamy, 1978) was followed. The experiment was repeated for three seasons during the year 2002 (January - February, April - May and July - August). Rearing was conducted in completely randomized block design with three replication of 100 larvae each after third moult. The eggs produced from control and treatment batches were further consigned for subsequent two generation to confirm the generation effect. The data recorded for three generation were analyzed statistically for the 10 important economic parameters *viz.*, Egg hatching (%), Weight of 10 grownup larvae (g) (WTG), Total larval duration (Days: Hours) (TLD), Fifth age larval duration (Days: Hours) (VLD), Cocoon yield (number)/10,000 larvae (YLN), Cocoon yield (kg)/10,000 larvae (YLW), Pupation rate (%) (PR), Single cocoon weight (g) (CWT), Single shell weight (cg) (SWT) and Shell ratio (%) (SR). The non-parametric tests statistics (Wilcoxon tests) was adopted by using the SPSS Inc. Statistical computer packages to compare the mean performance of treatment batches over the control.

Results and Discussion

The eggs released from first generation of experimental batch (45 days preservation schedule) showed poor hatching of eggs in 5 accessions *viz.*, Pure Mysore, Rong Diazo, GNM, WAI-1 and WAI-4. Similarly in the second generation the eggs showed poor hatching in 4 accessions *viz.*, A25, MU303, Daizo and PMX. The egg hatchability for practical use of these non-responded strains was recorded and subjected to non-parametric tests statistics by adopting Wilcoxon's model. The results revealed that there was a significant difference between control and treatment

Table 1. Mean performance and effect of egg hatchability for practical use (%) of nine non-responded multivoltine silkworm accessions

Race name	Egg hatching %		Z-value
	Control mean ± SE	Treatment mean ± SE	
Pure Mysore	87.1 ± 1.47	22.5 ± 1.31	2.882**
Rong Daizo	88.6 ± 1.90	14.6 ± 1.11	2.882**
A25	91.0 ± 1.50	22.7 ± 0.86	2.898**
GNM	91.8 ± 1.63	23.5 ± 0.92	2.892**
PMX	91.3 ± 1.57	24.5 ± 1.20	2.887**
WAI-1	89.0 ± 1.86	14.8 ± 1.25	2.887**
WAI-4	88.3 ± 2.27	7.1 ± 0.92	2.892**
Daizo	92.0 ± 1.43	15.8 ± 1.37	2.882**
MU303	90.0 ± 2.58	21.8 ± 1.25	2.887**
CD 5%	5.00	3.27	
ANOVA (F-value)	1.0 ^{NS}	26.14 ^{***}	

Note: Z value indicate Wilcoxon value.

***Significance at P < 0.001, **Significance at P < 0.01 and NS - Non significant.

batches for all the nine non-responded strains (Table 1). The nine accessions that did not respond to low temperature preservation might be due to low humidity level in the storage environment which resulted in poor hatchability of eggs (Shimizu *et al.*, 1994), besides, the relative tolerance level for low temperature is a racial characteristics (Yu *et al.*, 1993). Therefore only 54 accessions were experimented in the third generation. The mean performance of control and experiment for ten characteristics are presented in Table 2 and 3 respectively. The data were subjected to ANOVA and highly significant variability was recorded among the silkworm accessions for the eight parameters except for cocoon yield (number)/10,000 larvae, whereas, in the treatment batches all the nine parameters are found to be significantly varied.

The main objective of the study is to compare the rearing performance of the experimental batch (45 days egg preservation at 5°C) with that of control (30 days egg preservation at 5°C). Hence, the data recorded for three generation for both the batches were subjected to non-parametric tests statistics by adopting Wilcoxon's model. In all the accessions of experimental batch there was significant variation in total larval duration. Which was extended by 2 days over the control. The fifth age larval duration in 27 accessions decreased by one day compared to the control and quantitative traits *viz.*, single cocoon weight, single shell weight, shell ratio and pupation rate varied in 13 accessions (C. *nichi*, *Moria*, OS-616, B, KW2, A14DY, AP12, MW13, DMR, LMO, ZPN (SL),

Table 3. Mean performance for economic parameters of 54 multivoltine silkworm accessions reared after 45 days cold preservation of eggs

Race name	Egg hatching (%)		Weight of 10 grownup larvae (g)		Total larval duration (Days : Hrs)		Fifth age larval duration (Days : Hrs)		Cocoon yield (number)/10,000 larvae		Cocoon yield (kg)/10,000 larvae		Pupation rate (%)		Single cocoon wt. (g)		Single shell wt. (cg)		Shell ratio (%)	
	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE	mean ± SE	SE
C.nichi	88.5 ± 2.15		20.7 ± 0.60		23:01 ± 7.80		04:10 ± 4.39		9256 ± 360.60		9.5 ± 0.74		92.2 ± 2.61		1.06 ± 0.039		12.4 ± 0.003		11.8 ± 0.48	
GNP	89.6 ± 1.36		20.8 ± 0.64		23:08 ± 6.60		04:17 ± 1.95		8422 ± 561.19		9.4 ± 0.90		90.1 ± 1.31		1.01 ± 0.035		14.0 ± 0.007		14.1 ± 0.43	
Raj	87.3 ± 2.24		22.0 ± 1.27		22:22 ± 3.96		04:16 ± 1.59		9411 ± 344.98		9.7 ± 0.91		94.9 ± 0.57		0.99 ± 0.068		13.6 ± 0.008		13.9 ± 0.28	
Nistid (Y)	85.4 ± 1.21		17.3 ± 0.79		23:13 ± 6.33		05:03 ± 4.10		8822 ± 563.17		8.7 ± 1.14		93.3 ± 1.58		0.91 ± 0.053		12.3 ± 0.008		13.6 ± 0.31	
Nistid (W)	84.6 ± 0.65		21.0 ± 0.61		24:14 ± 24.30		04:15 ± 2.40		9411 ± 310.66		9.5 ± 0.54		95.9 ± 1.20		1.00 ± 0.027		13.5 ± 0.004		13.6 ± 0.27	
NK4	85.3 ± 0.82		24.7 ± 1.22		23:16 ± 6.53		05:01 ± 2.67		9478 ± 209.35		13.0 ± 1.35		89.1 ± 2.27		1.24 ± 0.030		16.7 ± 0.005		13.6 ± 0.47	
Cambodg	85.6 ± 2.34		20.9 ± 0.54		23:05 ± 6.11		04:22 ± 3.04		8533 ± 730.49		9.1 ± 0.98		88.9 ± 2.38		1.11 ± 0.031		15.8 ± 0.007		14.3 ± 0.58	
Sarapat	86.5 ± 1.48		19.7 ± 2.05		23:23 ± 6.56		05:12 ± 4.03		8400 ± 495.26		8.3 ± 0.45		90.6 ± 2.53		1.03 ± 0.058		15.1 ± 0.009		14.8 ± 0.39	
Moria	89.5 ± 1.56		21.1 ± 0.83		24:01 ± 6.92		05:05 ± 4.21		8936 ± 596.22		9.1 ± 0.61		91.4 ± 1.75		1.08 ± 0.026		15.3 ± 0.007		14.2 ± 0.44	
T. N. White	88.6 ± 2.87		19.8 ± 0.82		23:20 ± 7.84		05:02 ± 2.13		9467 ± 218.58		10.0 ± 0.92		91.6 ± 1.26		1.06 ± 0.043		15.4 ± 0.006		14.6 ± 0.25	
Hosa Mysore	88.2 ± 1.51		24.4 ± 1.73		23:12 ± 6.27		04:21 ± 1.16		9254 ± 361.97		10.9 ± 1.31		90.2 ± 6.84		1.28 ± 0.076		18.3 ± 0.013		14.3 ± 0.27	
Mysore Princess	95.0 ± 0.75		24.1 ± 1.33		23:19 ± 5.77		05:02 ± 3.98		8211 ± 581.53		10.2 ± 1.41		87.7 ± 4.66		1.21 ± 0.051		18.5 ± 0.007		15.4 ± 0.62	
Kolar Gold	93.2 ± 1.39		25.5 ± 1.25		24:05 ± 8.13		05:14 ± 2.12		9467 ± 180.28		11.6 ± 0.97		94.5 ± 1.28		1.31 ± 0.066		20.1 ± 0.011		15.5 ± 0.50	
Kollegal Jawan	87.9 ± 2.15		25.4 ± 1.58		23:20 ± 4.77		05:05 ± 4.45		9133 ± 305.51		11.5 ± 0.59		90.2 ± 3.43		1.29 ± 0.045		20.7 ± 0.006		16.3 ± 0.39	
MY1	92.0 ± 1.43		24.8 ± 1.57		23:20 ± 5.89		05:05 ± 2.11		9278 ± 300.36		10.1 ± 1.24		86.3 ± 4.90		1.26 ± 0.068		19.3 ± 0.008		15.6 ± 0.31	
P2D1	91.9 ± 1.19		23.6 ± 1.75		23:18 ± 5.42		05:04 ± 1.82		8744 ± 525.49		10.6 ± 0.87		94.2 ± 0.94		1.18 ± 0.072		17.7 ± 0.010		15.2 ± 0.21	
OS-616	91.8 ± 1.06		21.2 ± 1.05		23:07 ± 6.98		04:16 ± 1.75		9444 ± 233.40		10.5 ± 0.57		96.3 ± 0.69		1.08 ± 0.030		13.0 ± 0.006		12.1 ± 0.32	
G	86.2 ± 1.35		20.7 ± 1.02		24:02 ± 5.81		05:09 ± 4.18		9556 ± 212.21		11.0 ± 0.58		92.9 ± 1.35		1.10 ± 0.030		15.7 ± 0.007		14.2 ± 0.29	
Nistari	88.4 ± 1.18		22.3 ± 0.89		23:08 ± 8.49		04:17 ± 3.23		9289 ± 400.15		10.2 ± 0.74		95.8 ± 0.96		1.02 ± 0.032		13.7 ± 0.005		13.4 ± 0.18	
Nistari (M)	83.4 ± 1.03		22.7 ± 1.22		23:04 ± 6.02		03:21 ± 15.86		9300 ± 258.20		10.0 ± 0.57		95.1 ± 0.93		1.02 ± 0.037		13.5 ± 0.007		13.2 ± 0.22	
Nistari (P)	84.8 ± 0.74		21.9 ± 1.26		23:05 ± 6.65		04:22 ± 3.70		8952 ± 351.46		9.1 ± 0.72		91.0 ± 1.94		1.02 ± 0.065		13.3 ± 0.012		13.0 ± 0.39	
ZPN (SL)	85.8 ± 1.89		18.5 ± 0.93		24:04 ± 7.21		05:07 ± 2.73		8711 ± 387.82		7.7 ± 0.95		92.8 ± 3.00		1.02 ± 0.041		15.3 ± 0.005		15.1 ± 0.25	
CB5	86.6 ± 1.96		20.3 ± 0.90		23:14 ± 4.17		05:07 ± 1.86		9708 ± 116.96		8.6 ± 1.01		92.9 ± 1.54		1.04 ± 0.051		15.8 ± 0.007		15.4 ± 0.39	
KW2	88.9 ± 2.05		18.8 ± 0.59		23:21 ± 8.43		05:03 ± 1.45		9356 ± 299.12		9.2 ± 0.81		88.0 ± 5.43		1.00 ± 0.049		13.7 ± 0.008		13.8 ± 0.31	
M2	92.7 ± 1.51		21.0 ± 1.09		24:03 ± 8.19		05:10 ± 1.47		9541 ± 161.28		11.4 ± 0.69		92.3 ± 1.20		1.11 ± 0.038		16.3 ± 0.007		14.8 ± 0.57	
A23	87.8 ± 1.17		23.7 ± 0.98		23:16 ± 9.87		05:01 ± 2.91		9789 ± 153.16		11.4 ± 0.52		93.1 ± 1.28		1.15 ± 0.033		16.0 ± 0.005		14.0 ± 0.19	
OVAL	84.4 ± 0.88		18.4 ± 0.85		24:05 ± 6.57		05:09 ± 2.47		9444 ± 266.73		9.4 ± 1.40		94.9 ± 1.68		1.07 ± 0.025		15.0 ± 0.006		14.1 ± 0.23	
O	88.6 ± 1.52		24.5 ± 0.74		24:00 ± 7.50		05:09 ± 1.86		8600 ± 539.03		10.6 ± 0.62		95.8 ± 1.29		1.23 ± 0.059		18.1 ± 0.010		14.8 ± 0.34	

Table 4. Comparative performance for rearing parameters between control and treated batches (Wilcoxon's Non-parametric test statistics)

Race name	HAT Z-value	WTG Z-value	TLD Z-value	VLD Z-value	CWT Z-value	SWT Z-value	SR Z-value	YNO Z-value	YLW Z-value	PR Z-value
C.nichi	0.481	0.471	2.89**	0.596	2.13*	2.41*	0.280	1.310	0.120	0.290
GNP	1.283	1.53*	3.21***	1.080	1.020	0.280	1.940	0.530	1.060	0.240
Raj	1.925	1.410	3.30***	1.790	1.020	1.390	1.020	1.460	1.180	0.240
Nistid (Y)	1.925	2.24*	2.50*	2.50*	0.830	0.830	0.090	1.070	0.350	0.290
Nistid (W)	1.281	0.710	2.73**	2.89**	0.560	0.370	0.830	1.020	0.180	1.410
NK4	0.160	0.350	3.24***	3.07**	1.200	0.830	1.390	2.14*	1.530	0.820
Cambodg	1.764	3.06**	2.84**	3.05**	0.650	0.090	0.090	0.420	0.350	1.300
Sarupat	0.642	1.060	3.21***	0.780	0.090	0.280	0.280	0.830	2.47*	0.350
Moria	0.643	1.530	2.99**	1.620	2.31*	1.210	0.830	1.080	1.060	1.530
T. N. White	0.643	2.36*	2.50*	1.030	0.090	0.090	0.460	0.890	0.000	0.240
Hosa Mysore	0.480	1.180	2.63**	0.790	0.280	0.830	1.940	1.550	0.470	0.360
Mysore Princes	0.966	1.530	3.09**	1.070	0.280	0.550	1.200	1.160	0.820	0.240
Kolar Gold	0.321	0.940	2.96**	1.270	1.370	0.280	1.570	1.530	0.120	0.590
Kollegal Jawan	0.801	1.530	3.11**	0.830	1.200	0.830	0.280	1.070	0.120	0.120
MY1	0.480	1.060	2.90**	3.07**	0.460	1.200	0.090	0.830	0.290	1.000
P2D1	0.321	0.590	2.75**	2.88**	0.000	0.370	0.090	0.360	0.820	0.940
OS-616	0.962	2.0*	2.38*	3.12**	1.390	2.50*	2.50*	1.370	0.710	0.000
G	1.922	2.71**	3.15**	1.800	1.570	1.940	1.390	1.440	0.940	1.060
Nistari	0.482	1.890	2.27*	2.63**	0.090	0.090	0.650	1.090	0.470	0.830
Nistari (M)	1.283	1.060	2.17*	3.09**	0.090	0.930	1.940	0.540	0.650	1.530
Nistari (P)	1.125	1.410	2.50*	1.620	1.110	0.830	0.830	0.240	0.940	1.180
ZPN (SL)	1.286	0.240	2.76**	2.07*	1.200	1.390	1.390	0.470	1.180	2.24*
CB5	1.283	2.71**	2.62**	0.600	0.460	0.650	0.830	2.15*	1.290	2.12*
KW2	0.321	1.890	2.53*	0.500	0.650	1.400	2.31*	0.830	1.470	0.590
M2	0.642	2.47*	2.38*	0.060	0.830	1.300	0.830	0.770	0.060	0.590
A23	1.925	0.940	2.15*	0.720	0.280	1.580	1.940	2.07*	1.120	0.470
OVAL	1.604	2.83**	2.86**	2.41*	1.390	1.760	0.830	1.060	1.060	0.940
O	1.764	0.590	2.63**	0.730	0.650	0.180	1.760	0.120	0.240	1.180
M83(C)	1.123	1.060	1.790	2.51*	0.460	0.280	0.650	0.300	2.01*	0.120
B	0.322	3.18**	2.63**	1.930	2.120	2.50*	1.940	1.130	0.350	0.530
A14DY	1.60	0.24	2.50*	1.69	0.46	0.28	2.50*	0.83	1.59	0.59
A4E	1.12	0.35	2.20*	1.38	0.65	0.28	1.39	0.95	0.47	2.83**
PA12	1.60	0.71	2.50*	2.94**	0.83	0.09	1.94	0.71	0.12	0.35
AP12	1.76	1.06	2.14*	3.15**	0.28	0.28	2.13*	1.42	1.41	2.00*
A13	1.92	0.59	2.63**	1.33	1.39	0.28	1.57	0.65	0.47	1.30
PMS2	0.16	0.82	2.53*	1.02	0.65	0.46	1.02	1.06	0.94	0.24
MU-1	0.48	0.71	2.50*	3.23***	0.65	0.18	1.20	1.08	1.06	2.00
MU-11	1.29	1.08	2.62**	2.15*	0.68	0.68	0.84	0.84	1.08	0.92
MY23	1.28	1.06	3.22***	2.18*	1.02	0.83	0.65	0.18	0.12	0.24
MW13	1.29	0.47	3.21***	2.66**	0.37	1.02	2.49*	1.24	0.24	1.29
MHMP (W)	0.32	0.82	1.79	3.26***	0.65	1.48	0.28	2.19*	0.12	0.35
MHMP (Y)	0.64	0.71	3.12**	3.20***	1.39	1.39	1.57	2.10*	0.35	1.53
DMR	0.48	3.06**	2.18*	2.20*	1.39	1.76	2.13*	0.83	0.35	1.36

Table 4. Continued

Race name	HAT Z-value	WTG Z-value	TLD Z-value	VLD Z-value	CWT Z-value	SWT Z-value	SR Z-value	YNO Z-value	YLW Z-value	PR Z-value
P4D3	1.93	1.29	2.69**	2.74**	0.83	0.28	1.20	3.04**	1.65	1.30
LMP	0.80	3.18***	2.28*	2.05*	0.28	0.93	1.76	0.24	1.06	1.30
LMO	0.80	1.53	2.03*	1.22	0.83	0.83	2.13*	0.12	1.06	0.12
MY1 (SL)	1.93	2.12*	2.51*	2.29*	0.28	0.46	0.09	2.28*	0.94	0.35
PM (SL)	1.28	2.95**	3.20***	2.54*	0.83	1.21	1.20	0.94	1.77	0.00
BL23	1.76	2.47*	2.93**	2.18*	0.09	1.29	1.20	0.35	0.24	0.35
BL24	0.32	2.47*	2.51*	2.37*	0.83	0.46	0.09	1.52	1.60	0.96
MU520	1.61	1.53	3.25***	0.12	0.65	0.46	1.02	1.78	0.94	0.47
MU10	1.12	1.41	2.49*	1.21	0.28	0.46	1.76	0.36	1.29	0.24
TW × SK6 × SK1	0.32	1.53	2.17*	1.79	0.09	0.46	0.46	0.54	0.59	0.71
SK6 × SK1 × TW	0.97	0.47	1.78	0.55	1.95	1.20	1.39	1.06	2.47*	1.77

Note: Z value indicate Wilkcoxon value.

***Significance at $P < 0.001$, **Significance at $P < 0.01$ and *Significance at $P < 0.05$.

Abbreviation used: HAT = Egg hatching (%), WTG = Weight of 10 grownup larvae, TLD = Total larval duration, VLD = Fifth age larval duration, CWT = Single cocoon weight, SWT = Single shell weight, SR = Shell ratio, YNO = Cocoon yield (number)/10,000 larvae, YLW = Cocoon yield (kg)/10,000 larvae and PR = Pupation rate.

Table 5. Relative tolerance level of 63 multivoltine silkworm genotypes for the 45 days cold preservation of eggs

Sl. no.	No. of accessions	Name of the breeds	Nature of tolerance
1	41	GNP, Raj, Nistid (Y), Nistid (W), NK4, Cambodg, Sarupat, T. N. White, Hosa Mysore, Mysore Princes, Kolar Gold, Kollegal Jawan, MY1, P2D1, G, Nistari, Nistari (M), Nistari (P), M2, A23, Oval, O, M83 (c), PA12, A13, PMS2, MU-1, MU-11, MY23, MHMP (W), MHMP (Y), P4D3, LMP, MY1 (SL), PM (SL), BL23, BL24, MU520, MU10, TW x SK6 x SK1 and SK6 x SK1 x TW	Highly tolerant
2	13	C.nichi, Moria, OS-616, B, KW2, A14DY, AP12, MW13, DMR, LMO, ZPN (SL), CB5 and A4e	Moderately tolerant
3	9	Pure Mysore, Rong Diazo, GNM, WAI-1, WAI-4, A25, Mu303, Diazo and PMX	Highly susceptible

CB5 and A4e). The weight of 10 grownup larvae showed decreasing trend in 16 accessions, single cocoon weight in 2 accessions, single shell weight in 3 accessions, shell ratio in 7 accessions, cocoon yield (number)/10,000 larvae in 7 accessions, cocoon yield (kg)/10,000 larvae in 3 accessions and pupation rate in 4 accessions (Table 4 and 5). This result is in agreement with the findings of Meera Verma and Chauhan (1996) and Pandey and Upadhyay (2001).

The present study indicate that, the extension of the refrigeration period from 30 days to 45 days has prolonged the total larval duration in non-hibernating multivoltine silkworm accessions, which is positively correlated. The characters like weight of 10 grownup larvae, total and fifth age larval duration and cocoon yield (number)/10,000 larvae and cocoon yield (kg)/10,000 lar-

vae are mostly polygenic and therefore effected on the environmental influences. Thus, the variation obtained for those traits are not to be given much importance, since the main objectives of the silkworm genetic resources maintenance is to conserve their original racial characteristics which are oligogenic falling under the Mendelian law of inheritance. There is no alteration in the genetically controlled morphological characters *viz.*, larval pattern, haemolymph colour, cocoon colour and cocoon shape in 41 accessions due to prolonged preservation of eggs at 5°C for 45 days.

Considering the above said observation, it is recommended that except those silkworm accessions that failed to hatch after 45 days of preservation, the remaining multivoltine silkworm accessions may be preserved following 45 days cold preservation schedule, which are found to be

tolerant to long-term preservation of eggs for up to 45 days, as they have not showed any significant variation for both morphological as well as essential quantitative traits. Therefore, for those 41 accessions one rearing may be curtailed and 4 crops per year may be followed, which will reduce conservation cost considerably.

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