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Egg Diapause Induction in Multivoltine Silkworm *Bombyx mori for* Long-term Germplasm Preservation

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At present, multivoltine silkworm races reared five times per year involving huge manpower and rearing expenditure. Egg diapausing behavior is facultative in multivoltine and egg diapause was induced in selected multivoltine races by regulating temperature at 18°C, relative humidity 80% and photoperiod (6L : 18D) in the late stage silkworm rearing. The maximum percentage of egg diapause induction was recorded in Rong Diazo, Diazo and MW13 showed 94%, 93% and 92% respectively, whereas the races A14DY and OS-616 showed minimum diapause induction 15% and 18% respectively. The diapause induced multivoltine eggs were preserved up to six months by cold preservation schedule normally adopted for bivoltine. After three and six months egg preservation, the diapause induced layings were released and observed for hatching percentage, all races showed above 82 % of hatching except the race AP12, which showed only 78 % of hatching. This methods reduce the crop cycle, gives strong safety backup and preventing the genetic erosion. This study helps formulating a new conservation method for multivoltine silkworm germplasm.

Key words: *Bombyx mori L.*, Multivoltine, Diapause induction, Germplasm conservation

Introduction

The Central Sericultural Germplasm Resources Centre (CSGRC), Hosur has large collection of silkworm *Bombyx mori* L. germplasm resources and the gene bank of this centre holds 429 races of mulberry silkworm, representing 72 multivoltines, 337 bivoltines and 20 mutants. These collections include diverse germplasm materials represented for different geographical origin and shows diversity in morphological, reproductive, biochemical, growth and yield parameters.

Conservation of silkworm germplasm is scientific and technically demanding high skill and labour. Therefore, maintaining large collections of silkworm is costly affair, requiring huge resources. Besides, conservation of multivoltine silkworm germplasm through traditional practice of continuous rearing is risky as this not only invites diseases and pests, but there is chance of genetic erosion (Radhakrishnan, *et al.*, 2003).

Multivoltine silkworm races are preserved for 30 days in the cold storage and five rearing are conducted every year (Kumaresan *et al.*, 2004) whereas eggs of bivoltine races are preserved for 10-12 months in the cold storage and only one rearing is conducted in a year. Bivoltine races undergo hibernation (diapause), which helps to withstand low temperature of cold storage schedule of longer period. Generally, diapause is genetically controlled and also influenced by environment (temperature and photoperiod) and if this is induced in multivoltine, it becomes reversible when original environment acts upon. The objective of the study is to develop an alternative method for long term and cost effective conservation of multivoltine silkworm genetic resources by inducing diapause in non-hibernating silkworm germplasm. It also envisages developing protocols for diapause induction through photoperiod and temperature regulation (Nagayama and Yamamoto 1987).

Diapause occurs as an alternative developmental program in the life cycle and is accomplished by the dynamic change of developmental, behavioral and physiological events. The diapause program is expressed long before the

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developmental arrest appears (induction phase), and the program is again expressed long before any developmental resumption occurs to terminate diapause (termination phase). Expression of the diapause program is triggered by a particular set of environmental signals such as photoperiod, temperature and humidity. The received signals are transduced into endogenous chemical messengers, hormones, in the neuroendocrine organs. Finally, hormones bring about the phase change from development into diapause or *vice versa* through structural and functional changes in the target organs. Thus, diapause is such a sophisticated biological phenomenon that there is great diversity in the expression of the diapause syndrome among insect species (Lees, 1955; Saunders, 1982; Denlinger, 1985).

Incubation at lower temperature of 15°C gave non-diapause eggs and also at over 25°C produces diapause eggs. The temperature during the larval stages also influences the mode of diapause. The effects of temperature during earlier stages of larvae are the same as at the embryonic incubation stage (Tazima, 1978; Morohoshi, 1979) and light has almost the same effects as high temperature and darkness has results similar to those produced by low temperature. Illumination for 15 h per day is necessary for the eggs (during incubation) to produce moths that lay 100% diapause eggs (Kogure, 1933).

The tropical multivoltine silkworm exhibit the facultative type of diapause and the diapause/non-diapause relation is highly reversible in general (Murakami, 1989), which is under the control of a sex-linked gene, (npnd) as detected in a tropical multivoltine race, Cambodg (Murakami, 1990). The intermediate temperature regimen (20-23°C) during the late larval (4th & 5th) instars and pupal stages promote the production of diapause eggs in the progeny of several multivoltine races of *Bombyx mori* (Kobayashi *et al.*, 1986 ; Nakayama and Yamamoto 1987). Hence in this present research work an attempt has been made to induce egg diapause in selected multivoltine silkworm races.

Materials and Methods

Twenty multivoltine silkworm races are selected for the study. Selection of multivoltine silkworm germplasm has been made based on the information on the susceptibility status to 45 days of cold preservation at 5°C (Kumaresan *et al.*, 2003). To induce diapause in eggs of multivoltine silkworms the general methodology proposed by Kogure (1933) was followed to obtain diapause eggs in multivoltine i.e. incubation of layings at 25°C, 80%RH & 16L : 8D condition followed by rearing the 4th and 5th instar worms at 18-20°C with 6L : 18D. After III moult 300 worms in replication were kept for each race and rearing (Krishnaswami, 1978) was conducted for seven generations with the prescribed temperature, humidity and photoperiod.

Table 1. Egg preservation schedule for three and six month cold preservation

Temperature	3 month (Days)	6 month (Days)
25°C	10	20
20°C	4	4
15°C	4	4
10°C	4	4
5°C	50	90
2.5°C	20	60
5°C	1	1
15°C	3	3
25°C	Release	Release
Total	96	186

After obtaining cocoons, layings were prepared and the diapause responses of eggs were recorded. Then, the diapause-induced eggs (layings) were preserved for three and six months preservation schedule as followed for bivoltine eggs (Table. 1).

Upon time maturity of preservation schedule the diapause-induced layings were released and incubated at 25°C and 80% relative humidity for rearing to ascertain the reversibility of multivoltine characters in respect to survival and quantitative traits. During the experimental period, observations were made in respect of the following parameters and data were recorded race wise. The parameters include hatching percentage, 4th instar larval duration, 4th instar moulting duration, V instar larval duration, larval weight, cocoon weight, shell weight, shell ratio, larval survival percentage, pupal duration and fecundity etc. The data recorded for three generation on phenotypic and quantitative trait characters of diapause induced and control batches. The statistical analysis carried out by computer packages INDOSTAT were used to compare the mean performance of diapause induced and control batches.

Results and discussion

The result revealed that there was a significant variation on growth phase characteristics such as survivability, late age larval duration, moulting duration and cocoon characteristics of the selected races, which were reared at low temperature and less photoperiod (6L : 18D). In general, quantitative traits are polygenic in nature; the environ-

Table 2. Induction of diapause in multivoltine silkworm races in different Crop cycle

	Race Name	Egg diapause induction %							Average
		I crop	II crop	III crop	IV crop	V crop	VI crop	VII crop	
1	Pure mysore	55	10	5	48	30	21	16	26
2	Moria	60	59	65	69	71	65	57	64
3	C.nichi	33	63	6	41	43	12	25	32
4	Rong daizo	81	95	100	96	90	94	100	94
5	OS-616	17	5	15	24	7	16	40	18
6	ZPN (SL)	44	36	37	52	53	33	39	42
7	KW2	43	64	33	27	36	47	41	42
8	A25	29	27	32	30	45	16	17	28
9	B	14	7	12	7	29	4	8	12
10	GNM	80	80	83	96	78	73	93	83
11	A14DY	15	9	12	40	9	17	6	15
12	AP12	33	20	25	38	30	49	36	33
13	PMX	32	14	23	35	49	43	43	34
14	WAI-1	40	15	23	43	54	46	50	39
15	WAI-4	71	77	60	66	69	84	80	72
16	MW13	92	96	90	92	92	95	87	92
17	Daizo	90	96	96	90	91	100	87	93
18	DMR	22	12	5	31	16	38	22	21
19	LMO	61	39	44	68	63	70	82	61
20	MU303	36	41	21	28	40	39	27	33

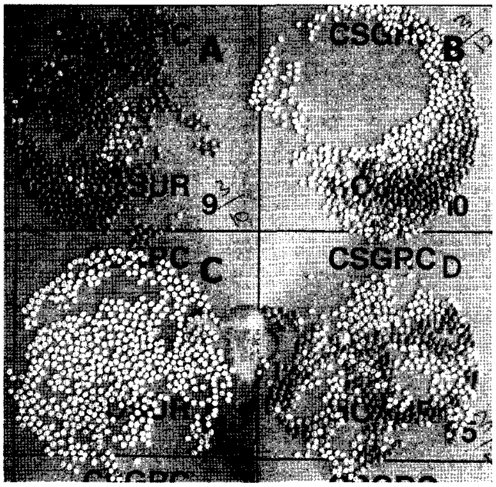
ment plays a vital role in the phenotypic expression of traits.

Variation in the diapause induction

In tropical multivoltine silkworm races, the diapause appearance is the facultative type with lot of intra variation in each race (Morohoshi, 1979; Murakami, 1989; Jayaswal *et al.*, 2001). In the present study, the differential response obtained for recovery of average egg diapause is ranged from 12 to 94% (Table 2). However, a positive trend was noticed that all the races selected in this present the study were more or less responded the egg diapause induction (Fig. 1). The hibernated layings were cold preserved for six months as followed for bivoltine egg preservation, to analyze the tolerance level of long-term cold preservation of multivoltine silkworm eggs.

Effect of low temperature on late age silkworm rearing

The mean performance of diapause induced and control rearing data for 11 characters were subjected to t-test (INDOSTAT) for comparative performance. The results showed that there is a significant variation in the all the characters. The diapause induced multivoltine eggs were kept at 5°C for 6 months cold preservation and incubated at 25°C. The hatching percentage of diapause-induced eggs was significantly different from control batch races C.nichi, Rong daizo, Ap12, Daizo and DMR. The diapause induced eggs maximum hatching percentage was 97.2% in the race C.nichi while in control it was 89.19% Similarly the minimum hatching percentage observed in race AP12 was 78.47% where as in control batch it was



A. Diapause eggs,
B. & D. Partial diapause eggs
C. Non-diapause eggs

Fig. 1. Diapause Induced eggs of Multivoltine Silkworms race (Moria).

Table 3. Mean performance of diapause-induced silkworm (*Bombyx mori*) rearing parameters after six months cold preservation and compared with control batches

Race Name	Hatching %			10 Larval wt (g)			4th Larval duration (Days: Hours)			4th Moulting duration (Days: Hours)		
	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value
Pure mysore	89.46±3.24	91.94±1.09	0.4766	20.38±1.94	24.10±0.84	0.1059	9.07±0.33	5.03±0.016	0.0051**	2.14±0.016	1.05±0.003	0.0003**
Moria	89.36±2.98	93.43±0.32	0.2966	24.08±0.76	29.23±0.61	0.0570	6.07±0.66	4.03±0.017	0.0552	2.03±0.017	1.03±0.017	0.0000**
C.nichi	97.20±0.65	89.19±0.59	0.0203*	27.46±2.13	24.88±0.39	0.2826	6.04±0.83	4.02±0.016	0.1051	1.08±0.166	1.02±0.016	0.0359*
Rong daizo	93.82±2.22	74.64±0.82	0.0228*	25.88±0.52	26.75±0.53	0.5639	6.04±0.33	4.03±0.017	0.0183*	2.12±0.027	1.03±0.016	0.0191*
OS-616	95.03±2.65	86.12±1.17	0.1110	27.98±0.64	24.47±1.20	0.1600	6.05±0.54	4.02±0.017	0.0641	2.03±0.017	1.02±0.017	0.0003**
ZPN (SL)	91.87±1.68	92.36±1.16	0.5048	28.41±0.33	20.76±0.32	0.0068**	6.08±0.52	4.02±0.014	0.0566	2.03±0.164	1.03±0.017	0.0131*
KW2	82.36±2.31	86.47±0.53	0.2810	25.84±0.31	26.69±0.23	0.7538	6.07±0.66	4.02±0.016	0.0573	2.02±0.167	1.02±0.066	0.0225*
A25	85.99±1.61	86.80±0.59	0.9045	28.20±1.39	26.82±0.36	0.2366	6.06±0.52	4.03±0.017	0.0566	2.02±0.016	1.03±0.017	0.0139*
B	90.13±2.36	94.41±1.23	0.2551	25.25±0.70	26.19±0.30	0.9349	5.07±0.27	4.02±0.066	0.0226	2.02±0.017	1.02±0.015	0.0000**
GNM	85.35±4.06	91.64±0.52	0.2595	27.34±1.46	27.45±0.24	0.9363	6.07±0.55	4.02±0.017	0.0633	2.04±0.127	1.02±0.017	0.0110*
A14DY	91.50±2.89	92.99±1.25	0.4500	24.75±1.48	23.66±0.51	0.4154	5.04±0.32	4.02±0.017	0.0460*	2.02±0.017	1.03±0.016	0.0003**
AP12	78.47±2.31	96.32±0.68	0.0135*	30.82±1.35	31.66±0.65	0.4650	6.07±0.63	4.02±0.015	0.0466*	2.03±0.016	1.03±0.017	0.0008**
PMX	90.01±2.32	92.66±0.67	0.2800	28.64±0.55	28.63±1.15	0.9700	5.07±0.33	4.02±0.017	0.0358*	2.03±0.066	1.02±0.017	0.0011**
WAI-1	94.67±1.26	96.19±0.23	0.7400	31.74±1.82	28.45±0.19	0.1820	6.05±0.56	4.02±0.017	0.0719	2.03±0.017	1.02±0.016	0.0143*
WAI-4	89.30±3.74	96.33±0.20	0.2000	28.38±0.95	28.81±0.53	0.7320	6.07±0.37	4.02±0.066	0.0174*	2.04±0.267	1.02±0.014	0.0076**
MW13	92.76±2.46	90.74±0.59	0.5000	29.98±1.30	32.66±0.62	0.1710	6.07±0.32	4.03±0.017	0.0138*	2.03±0.126	1.02±0.017	0.0120*
Daizo	96.90±0.36	88.43±0.87	0.0039**	18.90±0.97	18.62±0.65	0.8200	6.02±0.56	4.02±0.018	0.0667	2.02±0.017	1.02±0.027	0.0000**
DMR	93.84±1.56	88.28±0.67	0.0247*	26.70±1.20	29.31±0.53	0.1690	5.04±0.29	4.03±0.017	0.0477*	2.06±0.034	1.03±0.014	0.0006**
LMO	92.92±2.25	91.09±3.07	0.7500	28.22±0.66	27.63±0.22	0.4630	5.07±0.35	4.02±0.014	0.4226*	2.02±0.016	1.02±0.017	0.0000**
MU303	95.72±1.46	93.62±1.08	0.0700	29.72±1.52	24.63±1.13	0.5020	6.04±0.31	4.03±0.017	0.0195*	2.02±0.017	1.03±0.017	0.0003**

Table 3. *Continued...*

Race Name	5th Larval duration (Days: Hours)			Larval survival %			Cocoon wt (g)			Shell wt (g)		
	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value
Pure mysore	12.05±0.26	9.04±0.040	0.0055**	97.76±0.233	97.66±0.33	0.8580	1.02±0.08	1.29±0.042	0.4386	0.119±0.016	0.185±0.003	0.4226
Moria	9.05±0.26	6.02±0.017	0.0058**	91.56±0.882	91.00±0.58	0.6910	1.35±0.08	1.21±0.006	0.1757	0.200±0.003	0.184±0.001	0.1217
C.nichi	9.04±0.32	6.02±0.014	0.0078**	94.33±0.333	96.00±0.58	0.1990	1.62±0.02	1.20±0.009	0.1821	0.139±0.005	0.136±0.001	0.6068
Rong daizo	8.07±0.36	6.02±0.021	0.0161*	98.00±1.154	97.66±0.66	0.6660	1.34±0.07	1.23±0.006	0.6765	0.161±0.007	0.186±0.006	0.4623
OS-616	10.07±0.88	6.02±0.020	0.0332*	96.66±0.666	96.55±0.66	0.000**	1.28±0.04	1.32±0.159	0.6290	0.181±0.007	0.166±0.001	0.1620
ZPN (SL)	9.21±0.15	6.02±0.017	0.0018**	94.85±0.335	96.00±0.57	0.6660	1.29±0.03	1.17±0.018	0.3122	0.165±0.006	0.172±0.001	0.3917
KW2	10.07±0.17	6.02±0.016	0.0010**	93.00±1.154	96.33±0.35	0.2490	1.21±0.04	1.23±0.012	0.6049	0.173±0.005	0.187±0.001	0.0533
A25	8.05±0.29	6.03±0.017	0.0121*	96.65±0.882	95.55±0.33	0.4220	1.43±0.05	1.34±0.006	0.1662	0.190±0.002	0.182±0.001	0.0462*
B	9.08±0.04	6.02±0.011	0.0001**	92.33±1.201	94.00±1.00	0.0377*	1.21±0.07	1.17±0.002	0.1010	0.142±0.004	0.160±0.003	0.0341*

Table 3. *Continued...*

GNM	10.07±0.17	6.03±0.018	0.0016**	94.85±0.335	96.00±0.57	0.6660	1.34±0.06	1.23±0.001	0.6963	0.200±0.007	0.188±0.012	0.8140
A14DY	8.07±0.33	6.03±0.017	0.0164*	96.00±0.577	96.33±0.88	0.6075	1.23±0.07	1.08±0.002	0.3380	0.174±0.021	0.162±0.001	0.0926
AP12	8.09±0.40	6.02±0.004	0.0191*	95.65±0.882	96.33±0.66	0.1850	1.41±0.04	1.23±0.001	0.2730	0.200±0.019	0.202±0.004	0.2044
PMX	9.04±0.127	6.02±0.017	0.0018**	90.00±1.527	91.65±0.33	0.3370	1.40±0.04	1.53±0.002	0.3700	0.200±0.003	0.184±0.001	0.1217
WAI-1	8.04±0.355	6.02±0.016	0.0224*	93.65±0.882	95.66±0.33	0.0742	1.46±0.07	1.27±0.002	0.1150	0.210±0.003	0.196±0.003	0.0075**
WAI-4	9.08±0.814	6.02±0.015	0.0402*	95.65±0.333	97.00±1.00	0.6070	1.26±0.11	1.37±0.006	0.2850	0.172±0.006	0.191±0.002	0.0790
MW13	9.04±0.355	6.03±0.020	0.1103*	96.00±0.577	96.33±0.88	0.6075	1.24±0.20	1.68±0.006	1.0000	0.195±0.025	0.288±0.023	0.5626
Daizo	9.04±0.540	6.02±0.017	0.0287	96.65±0.333	95.66±0.88	0.4770	0.95±0.07	0.97±0.006	0.5560	0.088±0.013	0.136±0.003	0.1946
DMR	9.017±0.017	6.03±0.017	0.0000**	94.66±0.666	96.00±0.57	0.0572	1.28±0.65	1.30±0.006	0.7666	0.178±0.010	0.181±0.002	0.6269
LMO	9.017±0.017	6.02±0.018	0.0000**	93.33±1.855	94.33±0.88	0.4220	1.32±0.04	1.19±0.000	0.0517	0.180±0.010	0.163±0.002	0.0261*
MU303	8.074±0.373	6.02±0.017	0.0174*	98.00±1.154	97.68±0.33	0.7410	1.45±0.06	1.27±0.006	0.6925	0.197±0.012	0.192±0.002	0.7902

Table 3. *Continued...*

Race Name	Silk Ratio %			Pupal duration (Days: Hours)			Fecundity		
	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value	T Mean±S E	C Mean±S E	t- value
Pure mysore	11.55±0.65	14.48±0.70	0.6212	15.03±0.33	11.06±0.30	0.0082**	404.07±37.05	544.33±6.33	0.0452*
Moria	14.02±0.70	14.99±0.90	0.3968	14.03±0.33	11.03±0.33	0.0351*	358.84±8.46	434.33±13.91	0.0430*
C.nichi	11.02±0.55	11.32±0.02	0.6536	13.07±1.20	11.02±0.02	0.1606	371.96±24.56	454.00±16.92	0.0466*
Rong daizo	12.06±0.20	16.70±0.04	0.1318	14.03±1.20	11.02±0.02	0.1126	441.38±16.87	674.83±2.05	0.0176*
OS-616	14.15±0.17	12.89±1.34	0.4733	14.00±0.58	11.03±0.33	0.0942	490.67±63.94	453.60±4.90	0.6946
ZPN (SL)	12.75±0.24	14.69±0.24	0.3576	14.00±0.58	11.03±0.21	0.0153*	368.67±29.87	616.84±3.61	0.0339*
KW2	14.28±0.28	16.23±0.06.	0.1492	13.07±0.67	11.03±0.33	0.1181	378.22±39.13	431.33±3.53	0.2756
A25	14.97±0.97	13.64±0.05	0.5704	14.00±0.58	11.02±0.02	0.0372*	397.00±21.36	483.65±4.10	0.0697
B	11.21±0.32	12.75±0.25	0.2771	13.07±0.88	11.03±0.33	0.0771	383.33±4.06	440.50±3.04	0.0052**
GNM	14.95±0.23	14.95±0.10	0.6221	15.00±0.58	11.03±0.21	0.3508	352.78±47.16	326.33±4.33	0.6206
A14DY	14.15±0.84	14.96±0.04	0.0556	14.03±1.20	11.06±0.19	0.2039	345.87±39.75	479.33±8.08	0.0520
AP12	14.09±0.95	16.33±0.35	0.1528	14.07±0.88	11.03±0.33	0.0377*	284.11±23.53	363.00±1.73	0.1100
PMX	14.29±0.40	13.64±0.07	0.0605	14.07±1.33	11.07±0.30	0.1885	368.33±29.20	367.00±2.00	0.9665
WAI-1	14.45±0.56	16.40±0.25	0.688	14.07±0.88	11.07±0.31	0.1217	487.83±17.82	430.66±4.48	0.0830
WAI-4	13.82±0.88	13.96±0.16	0.9507	14.00±0.00	11.07±0.33	0.0361*	468.83±50.82	490.60±3.32	0.6860
MW13	15.86±0.63	18.26±0.19	0.7185	15.00±0.00	11.07±0.66	0.0377*	465.24±25.38	468.66±1.85	0.8980
Daizo	9.18±0.65	13.89±0.22	0.967	14.00±1.00	11.00±0.33	0.0942	430.76±33.19	394.33±2.35	0.3740
DMR	13.94±0.14	13.92±0.13	0.896	14.03±1.20	11.00±0.27	0.1885	380.87±13.13	686.35±3.18	0.0061**
LMO	13.67±0.39	13.99±0.13	0.1361	14.03±1.20	11.02±0.02	0.1126	442.11±29.94	404.17±5.64	0.3960
MU303	13.61±0.45	15.16±0.16	0.6215	14.07±0.33	12.03±0.67	0.0198*	613.13±5.96	404.00±3.47	0.0005**

***Significance at $P<0.001$ **Significance at $P<0.01$ *Significance at $P<0.05$ No Significance / T-Treated, C-Control, t- t paired value, SE-Standard error

96.32%, which indicates that these races could be preserved up to six months similar to bivoltine egg preservation.

The results revealed that there was no significant variation in survivability of diapause induced and control batches and most of the selected multivoltine races exhibited same trend of survivability ranged from 91 to 98% without any detrimental effect, which emphasize that the tropical races possess potentiality to survive even under the low temperature similar to temperate races.

The rearing at lower temperature resulted increase in the fourth instar larval duration, fourth moulting duration and fifth instars larval duration and all these periods significantly increased in diapause induced silkworm races. In general, the late larval duration in silkworm is about 4 to 4 1/2 days in 4th instar and 6 to 7 days in 5th instar; and the fourth moult duration is around 1 1/2 days. In the present study, the 4th instar duration was found that increased about 3 days more than that of control rearing due to low temperature in the rearing environment. The hardy multivoltine race Pure Mysore is unique for its longer larval duration also showed further increment about 4 days in 4th instar duration in the treated batch, whereas the races A14DY, B, PMX, DMR and LMO showed less larval period when compared to Pure Mysore. This indicates the differential response of races even in the same stress condition. Similarly the fifth instar duration also increased about 2 to 4 days more than that of control (Table 3). Though increased in larval duration is influenced the larval weight but does not have any impact on silk ratio percentage of individual silkworm races. This may be due to slow down of physiological and hormonal activity under low temperature (Morohoshi, 1979), with out any adverse effect on growth of silkworm.

Since the late stage silkworms were reared at 18°C the larval weight was increased in diapause-induced silkworms. The 10 larval weight of diapause induced silkworm 28.41 g in the race ZPN (SL) it was significantly reduced in the control batches of 10 larval weights was 20.76 g. In most of the silkworm races shows similar trend of larval weight in the diapause induced and control batches. The cocoon characters are mostly related with the raw silk production and yarn quality. The present study indicated that there was increasing trend in pupal weight, which might be due to more consumption of food during the prolonged larval duration, resulted in significant variation on cocoon weight, as reported by Sureshkumar *et al.,* (2000). But in the case of silk ratio, which did not show any alteration when compared to control. It indicates that the quantity of silk shell (weight) is highly heritable, unique to race and more stable even under the fluctuating environmental condition (Jayaswal, 1996). It indicates that the treatment had no uniform profound influence on silk synthesis of all the races but the difference was due to varietal response.

There was significant difference in the pupal duration. The pupal duration increased about 2 to 4 days in diapause-induced batches in all the races when compared with control. Contrary to this finding the fecundity was decreased in the 14 races and increased in 6 races in diapause induced batches compared over the control batches. After seventh generation diapause induced eggs were released and conducted the normal rearing for confirming the reversible of its original characters. The results showed as in control.

This study draw a noteworthy finding that the tool of induction of diapause in multivoltine races could be adopted for conservation of those multivoltine silkworm races, which respond positively as this type of facultative diapause is a reversible one when the original environment acts upon them. Therefore, it may be concluded that the twenty races experimented under this study could be preserved successfully for three and six month at low temperature by adopting the methodology for germplasm conservation. These results enlighten the possibility of the methodology for germplasm conservation as the traits, which are highly heritable and genetically stable performance under low temperature besides no change in phenotypic expression.

The result inferred that, by adopting this technique three rearing could be curtailed from the present five crops per year for conservation of multivoltine silkworm races, subsequently a considerable amount of manpower, expenditure and genetic erosion could be minimized.

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