Preservation of Acid Treated Bivoltine Eggs in Silkworm Bombyx mori L.

K. L. Rajanna*, P. Jayarama Raju†, C. J. Prabhakar‡ and C. K. Kamble§

Silkworm Seed Technology Laboratory, Kodathi, Bangalore-560035, India.
†CSRTI Research Extension Centre, Bidaraguppe, Bangalore, India.
‡Central Muga Eri Research & Training Institute, Lahdoigarh, Assam, India.
§Central Sericultural Research & Training Institute, Mysore, India.

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The hybrid (CSR2 x CSR4) eggs treated with acid were taken up for the study with an objective to develop long-term preservation schedule. The hybrid eggs obtained with two mating duration (3 h and 6 h) and oviposition period (6 h and 24 h) with two age groups of eggs (24 h and 36 h) were treated with Hydrochloric acid. These eggs were subjected to preservation at 5°C in single step refrigeration and at 5°C and 2.5°C under double step refrigeration from 10~120 days. These eggs were released from the cold storage as per the specified durations and incubated at standard conditions and allowed 2 h for hatching at 450 lux light. Hatchability was found to be significantly higher or on par with the control in three treatments (T1, T2 and T4) where the eggs are preserved continuously at 5°C up to 30 days. However under double step refrigeration, hatching was not significantly affected in 20+60 day’s combination of T1 treatment up to 80 days. Bioassay studies of the promising treatment i.e., T1 with (20+60) days indicated that early stage loss and cocoon yield was found to be on par with the control. Hence this treatment was recommended for preservation of acid treated new bivoltine hybrid layings. Details of the hatchability and rearing performance of long term preservation of acid treated eggs are discussed.

Key words: Bivoltine, Acid treatment, Preservation, Hatchability.

Introduction

The preservation of hibernated silkworm eggs have been extensively studied and methods for preservation of eggs for short and long term without causing any physiological injury to the eggs have been investigated (Vemananda Reddy, 2004). The safe period of cold storage of silkworm eggs is dependent on the stage of embryo (Yamashita and Yaginuma, 1991). Embryogenisis in silkworm needs the best eco-physiological condition to retain the vigor of newly hatched larva (Yamashita and Hasegawa, 1985) and the water evaporation rate is negligible in diapause eggs than non-diapause eggs which enables them to survive for long term (Kim et al., 1981). Further, the conversion of glycogen into polyols (sorbitol and glycerol) at the time of initiation of diapause and reconversion of polyols into glycogen at the time of termination of diapause, which is utilized, for embryonic development is extensively studied (Chino, 1957a, 1957b, 1958, 1960; Furusawa et al., 1989; Yaginuma and Yamashita, 1977; Yamashita and Yaginuma, 1991). The requirement of bivoltine eggs after 50 days of oviposition can be met by chilling followed by hydrochlorisation technique.

Many workers have attempted refrigeration of non-diapause eggs to postpone hatching (Chen and Hsieh, 1993; Datta et al., 1972; Hayashi, 1991; Hurakadli et al., 1998; Kumaresan et al., 2004; Rajanna et al., 2006a, 2006b, 2008; Shen et al., 1988; Shimazu, 1973; Tayade et al., 1987). The levels of glycogen and its polyols (glycerol and sorbitol) showed fluctuations under low temperature (Furusawa, 1987a, 1987b) indicating a possible adaptation to cold stress. Presently acid treated bivoltine eggs can be refrigerated for a maximum period of 20 days. In tropical conditions, many times it is inevitable to postpone hatching beyond 20 days due to various reasons mainly seasonal changes and leaf availability. Moreover information
is scanty with respect to preservation of artificial non-hibernated eggs and there is no effective schedule to meet the demand of bivoltine eggs from 30–60 days. Hence, an attempt has been made to evolve a suitable long-term preservation technique for acid treated bivoltine eggs of new bivoltine hybrids.

Materials and Methods

Moths of CSR2 and CSR4 emerged from quality seed cocoons were given pairing (CSR2 x CSR4) for 3 h and 6 h. After depairing, the female breeds were allowed for oviposition at standard conditions of 25 ± 1°C temperature and 75 ± 5% RH for 6 h and 24 h. The following four treatments were considered for the preservation study.

Treatments

- **T1**: 6 h pairing, 6 h oviposition and 24 h embryonic age
- **T2**: 6 h pairing, 6 h oviposition and 36 h embryonic age
- **T3**: 3 h pairing, 24 h oviposition and 24 h embryonic age
- **T4**: 3 h pairing, 24 h oviposition and 36 h embryonic age

**Control**: 3 h pairing, 24 h oviposition and 36 h embryonic age (without refrigeration)

The zero hour was fixed after 6 hours from the time of depairing in T1 and T2 treatments and 8 hours after depairing in case of T3 and T4 treatments. The eggs of 20 h age were treated with HCL of specific gravity of 1.075 (recorded at 15°C) at 46.1°C temperature for 5 minutes to prevent the eggs from entering into diapause. Based on the zero hour, the embryonic age of the eggs was calculated and the eggs are preserved at 5°C at 24 and 36 hours embryonic age.

**Schedule of Preservation**

<table>
<thead>
<tr>
<th>Single step refrigeration</th>
<th>Double step refrigeration</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>25°C</td>
</tr>
<tr>
<td>15°C (3h)</td>
<td>15°C (3h) 15°C (2 days)</td>
</tr>
<tr>
<td>5°C</td>
<td>5°C 25°C</td>
</tr>
<tr>
<td>(up to 120 days)</td>
<td>(20 days) (60 days)</td>
</tr>
</tbody>
</table>

The eggs were preserved at 5°C continuously up to 120 days. These eggs were released at an interval of 10 days under four treatments i.e. T1, T2, T3 and T4 to study the effect of treatment on hatchability. In another set of study, acid treated bivoltine eggs were preserved under double step refrigeration for 20, 40 and 60 days of initial preservation at 5°C followed by an intermediate temperature of 15°C for two days. After confirming the critical embryonic stage (longest embryonic stage/Hei-B stage) the eggs are re-refrigerated at 2.5°C for 60, 40 and 20 days (double refrigeration) respectively for a total period of 80 days. Both at the time of preservation and release, the eggs are compulsorily passed through 15°C for 2 hours to avoid cold injury and thermal shock. The released eggs were incubated at 25 ± 1°C and 75 ± 5% RH and recorded hatching percentage. The experiment was repeated three times with three replications of 20 dfls each in each treatment. Bioassay studies were conducted with promising treatment to study the effect of long term preservation on important rearing and reeling parameters such as early stage loss (ESL), effective rate of rearing (ERR), cocoon weight, shell weight, shell ratio, filament length, non breakable filament length (NBFL), denier, renditta, and reelability.

Results and Discussion

Data of the hatchability of the eggs preserved continuously at 5°C under four treatments is depicted in (Fig. 1). Data revealed significant difference between treatments and durations. However there was no considerable difference in hatching between control and T1, T2 and T4 up to 30 days of preservation. As the eggs laid by the moths under long pairing duration (6 h) and short oviposition period (6 h) were more uniform in embryonic age, which resulted in better hatchability. However, the hatching percentage in T3 was significantly affected which might be due to the preservation of young age of embryo (24 h). Hatchability of the eggs preserved under double step refrigeration is presented in (Figs. 2 and 3). The hatchability was significantly higher in double step refrigeration when compared to single step refrigeration at 60 and 80 days in all four treatments. However, when compare to control the hatchability was not significantly affected in T1 with (20 + 60) day’s combination. The hatchability was found to be more than 90% in T2 and T4 with (20 + 60)

![Fig. 1. Hatching of acid treated CSR2 xCSR4 eggs preserved at 5°C.](image)
day’s combination and it was significantly affected in T2, T3 and T4 with 40 + 40 and 60 + 20 day’s combinations. The hatchability of the eggs refrigerated up to 60 days under double step refrigeration was not significantly affected in T1 with 20 + 40 and 40 + 20 day’s combinations and T4 with 20 + 40 day’s combination. However hatchability was significantly affected in T3 under double step refrigeration also. It is well-established fact that exposure of insect eggs to low temperature produce anti-freeze substance i.e., polyols such as glycerol and sorbitol (Steel, 1981). Silkworm eggs are cold stored at different low temperatures 2 or 3 phases in order to prolong the safe period of preservation (Yakoyoma, 1973). The increased cold tolerance with >90% hatching in double step refrigeration (5 and 2.5°C) is due to retention of polyols for long period compared to single step preservation at 5°C (Furusawa et al.; 1992).

The results of the bioassay studies are presented in (Table 1). The performance of T1 with 20 + 60 day’s combination, which has shown promising in all the three trials, is compared with untreated control. The results indicated that there was no significant difference between treatment and control with respect to hatchability, early stage loss, single cocoon weight and single shell weight but the shell ratio is significantly higher than control indicating that there was no adverse effect of cold storage on hatching and crop performance in the eggs of T1. Important reeling parameters such as filament length, non-breakable filament length (NBFL), denier, reelability and renditta were not significantly affected (Table 2).

These results of hatchability, rearing and reeling performance in T1 eggs have made us to recommend the schedule of preservation in bivoltine seed production especially in tropical conditions. Hence double step refrigeration and it was significantly affected in T2, T3 and T4 with 40 + 40 and 60 + 20 day’s combinations.

### Table 1. Effect of 80 days preservation of acid treated eggs on rearing parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hatch (%)</th>
<th>ESL (%)</th>
<th>Larval wt. (g)</th>
<th>ERR By No.</th>
<th>ERR By Wt. (Kg)</th>
<th>SCW (g)</th>
<th>SSW (g)</th>
<th>SR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/6/24(20 + 60)</td>
<td>93.93</td>
<td>15.02</td>
<td>38.91</td>
<td>8678</td>
<td>15.03</td>
<td>1.68</td>
<td>0.350</td>
<td>21.05</td>
</tr>
<tr>
<td>Control</td>
<td>94.51</td>
<td>14.73</td>
<td>40.07</td>
<td>8750</td>
<td>15.41</td>
<td>1.70</td>
<td>0.350</td>
<td>20.33</td>
</tr>
<tr>
<td>CD @ 5%</td>
<td>NS</td>
<td>NS</td>
<td>0.55</td>
<td>66</td>
<td>0.25</td>
<td>0.02</td>
<td>NS</td>
<td>0.72</td>
</tr>
</tbody>
</table>

### Table 2. Effect of 80 days preservation of acid treated eggs on reeling parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FL (m)</th>
<th>NBFL (m)</th>
<th>Denier (d)</th>
<th>Reelability (%)</th>
<th>Renditta</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/6/24(20 + 60)</td>
<td>982</td>
<td>756</td>
<td>2.60</td>
<td>83.57</td>
<td>6.7</td>
</tr>
<tr>
<td>Control</td>
<td>991</td>
<td>832</td>
<td>2.70</td>
<td>84.56</td>
<td>6.4</td>
</tr>
<tr>
<td>CD @ 5%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
peration technique could be utilized to increase shelf life of the acid treated new bivoltine hybrids eggs.

References


