*Journal of Advances in Biology & Biotechnology*

*Volume 27, Issue 6, Page 199-208, 2024; Article no.JABB.116877*
*ISSN: 2394-1081*

# Evaluation of Reproductive and Growth Performance in New Breeds and Hybrids of Bivoltine Silkworm (*Bombyx mori* L.)

**Thrilekha, D [a*], Manjunatha Gowda [a], Pradip G.D [a], P. Harshita Mala [a], Chethankumar D.S [a] and J. Seetharamulu [b++]**

[a] *Department of Sericulture, College of Agriculture, UAS, GKVK, Bengaluru – 560065, India.*
[b] *APPSRDI, Hindupur, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Author TD executed the research, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MG planned the study and arranged for the silkworm breeds. Authors PGD, PHM and CDS supported in silkworm rearing, managed the analyses of the study and the literature searches. Author JS supported in designing the breeding schemes. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JABB/2024/v27i6879

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/116877

***Original Research Article***

***Received: 01/03/2024***
***Accepted: 05/05/2024***
***Published: 09/05/2024***

## ABSTRACT

This study aimed to evaluate the reproductive and growth performance of recently developed bivoltine silkworm parental breeds and their hybrids to improve silk production and ensure the long-term sustainability of sericulture. Utilizing five thermotolerant pure breeds (B1, B2, B4, B6 and B8) and four popular CSR breeds (CSR2, CSR27, CSR6 and CSR26), six foundation crosses and eight

[++] *Director;*
**Corresponding author: E-mail: thrilekhareddydurgam@gmail.com;*

double hybrids were developed and evaluated for ten traits. Results showed that B1 exhibited superior performance in fecundity (524.00), hatching percentage (98.67%), dead eggs (1.33%), pupal weight (1.31 g), pupation rate (96.84%) and cumulative survival index (CSI) (93.84%). The foundation cross B1 × B2 excelled in fecundity (553.67), larval mortality (2.67%), pupal weight (1.53 g), ERR (97.33%) and CSI (93.27%) while FC2 performed better in hatching percentage (97.60%), dead eggs percentage (2.40%), fifth instar larval duration (157.21 h), fifth instar larval weight (35.64 g/10 larvae) and pupation rate (96.33%). Among the double hybrids, (B1 × B2) × (FC1) demonstrated superior performance in fecundity (614.33), larval mortality (1.41%), pupal weight (2.06 g), ERR (98.59%) and CSI (97.24%). FC2 × FC1 excelled in larval duration (141.11 h), fifth instar larval weight (51.96 g/10 larvae) and pupation rate (98.94%). Double hybrids showed significant improvements compared to parental breeds and foundation crosses, indicating potential for field testing and further development. These results indicate the potential of using the newly identified breeds and foundation crosses in silkworm breeding, particularly in developing new double hybrids as an alternative to established CSR breeds and hybrids.

*Keywords:* *Bombyx mori; double hybrids; foundation crosses; parental breeds; reproductive performance; silkworm.*

## 1. INTRODUCTION

Silkworm rearing has been a cornerstone of sericulture, contributing significantly to the global textile industry. Mulberry silkworm (*Bombyx mori* L.) is an important economic insect in the commercial production of silk, a natural fiber prized for its luster, strength and breathability. India, being a tropical country, predominantly relies on multivoltine × bivoltine hybrids, the resulting raw silk often falls short of international quality standards compared to bivoltine x bivoltine hybrids [1]. Emphasizing bivoltine sericulture is essential for meeting these benchmarks and improving silk production. In recent years, efforts have intensified to enhance silk production and ensure the sustainability of sericulture through the development of new bivoltine silkworm breeds and hybrids. Bivoltine silkworm breeds offer an efficient means to increase silk yield. Nevertheless, with climate change presenting challenges such as fluctuating temperatures, there is a growing need for silkworm breeds and hybrids capable of thriving under these conditions while maintaining high cocoon quality. Traditional breeding methods have successfully produced productive silkworm breeds in India. However, pure silkworm races often exhibit a negative correlation between high cocoon shell ratio and low pupation rate, necessitating careful management [2]. While hybrids offer the potential for high yields, obtaining a sufficient number of eggs from parent cocoons remains a challenge. Double hybrid production through foundation crosses (dumbbell × dumbbell or oval × oval) has emerged as a solution for increased egg numbers and recovery. These foundation crosses are easier for farmers to manage and result in healthier pupae compared to single parental lines [3]. However, continuous inbreeding leads to the deterioration of parental stock performance, necessitating the timely evolution of new, productive breeds and hybrids to replace weaker counterparts [4]. Introducing the new season and region-specific silkworm hybrids ensures higher gain for both the primary producers and reelers alike. Evaluating the reproductive and growth performance of new bivoltine breeds is vital for selecting high performing breeds and hybrids that can enhance silk production efficiency and promote a sustainable sericulture industry. Understanding the genetic diversity and performance of various silkworm strains is essential for breeders aiming to develop superior varieties with desirable traits. This study focuses on ten key traits, including fecundity, hatching and dead egg percentage, larval mortality, fifth instar larval duration and weight, pupal weight, pupation rate, ERR and cumulative survival index (CSI). Assessing the performance of both parental breeds and their hybrid progeny is crucial for identifying promising individuals with superior traits, thus facilitating their selection for further advancement in breeding programs.

## 2. MATERIALS AND METHODS

### 2.1 Silkworm Breeds

Five thermotolerant bivoltine silkworm breeds *viz.,* B1, B2, B4 (oval cocoon spinning breeds) B6 and B8 (peanut cocoon spinning breeds) and four productive CSR parental races (CSR2, CSR27, CSR6 and CSR26) were utilized for the preparation of hybrids. Accordingly, $F_1$'s of four oval cocoon spinning foundation crosses (FCs) *viz*., B1 × B2, B1 × B4, B2 × B4 & FC2 (CSR2 × CSR27) and two peanut cocoon spinning FCs *viz*., B6 × B8 & FC1(CSR6 × CSR26) were developed. By using six foundation crosses, eight double hybrids were developed *viz.,* (B1 × B2) × (B6 × B8), (B1 × B4) × (B6 × B8), (B2 × B4) × (B6 × B8), (FC2) × (B6 × B8), (B1× B2) × (FC1), (B1 × B4) × (FC1), (B2 × B4) × (FC1) and FC2 × FC1.

## 2.1 Silkworm Rearing and Hybridization

The disease-free layings of B series lines were procured from CSRTI, Mysore and CSR breeds obtained from NSSO. The disease-free layings of bivoltine foundation crosses and double hybrids were prepared at the Department of Sericulture, University of Agricultural Sciences, GKVK, Bengaluru during 2022-2023 by standard grainage technology [5]. Foundation crosses were prepared by using oval × oval and dumbbell × dumbbell breeds. Oval and dumbbell FCs were used (oval × oval) × (dumb-bell × dumb-bell) in double hybrids preparation. Deflossed cocoons were cut at one end to remove the pupae for sex determination, based on morphological characteristics such as markings and size. Pupae of different pure races and foundation crosses, segregated by sex, were preserved in separate trays. Moths were induced to emerge simultaneously by refrigerating male pupae on the 7th day of spinning. Male moths emerged before females and were refrigerated for later use. Healthy females of desired races/hybrids were placed in trays, paired with healthy males and kept undisturbed for copulation. After mating, females were allowed to lay eggs. Mated females were placed on egg sheets to lay eggs for 24 hours in darkness. Egg cards were treated with 2% formaldehyde solution for 15 minutes, washed and dried to remove surface contamination before acid treatment. Hydrochloric acid was heated to 46.1ºC and eggs aged 16-20 hours were dipped for 5-6 minutes to break diapause.

The diseased free layings of all the pure lines and hybrids were Incubated with optimum environmental conditions of 25±1°C temperature and 75±5% relative humidity until the 8$^{th}$ day followed by black boxing to achieve uniform hatching. Newly hatched larvae were brushed with freshly chopped mulberry leaves. Silkworm rearing was done by following the suitable rearing method [5]. During rearing, three feedings with fresh V-1 mulberry leaves were provided to all the worms till onset of spinning. All the parents and hybrids were reared following a completely randomized design with three replications each and 200 larvae were maintained in each replication after 3$^{rd}$ moult. At the end of 5$^{th}$ instar, the spinning larvae were collected manually and transferred to bamboo mountages. The cocoons were harvested on 6/7$^{th}$ day of mounting and assessed the following day for reproductive parameters such as fecundity (number), hatching rate (%), dead eggs (%) and pupation rate (%), along with growth parameters including larval mortality (%), fifth instar larval weight (g/10 larvae), fifth instar larval duration (h), pupal weight (g), Effective rate of rearing (%) and cumulative survival index (CSI) (%). were assessed for all parental breeds and hybrids. The data obtained with respect of different traits from two rearings was pooled and analyzed statistically using OP STAT software. Duncan's Multiple Range Test (DMRT) was used to compare the mean values of the study [6]. To assess the overall survivability of silkworms, cumulative survival index (CSI) [7] was calculated using the formula,

$$CSI=[(ERR/100) \times (pupation\ rate /100)] \times 100$$

where; CSI- Cumulative Survival Index; ERR- Effective Rate of Rearing

# 3. RESULTS AND DISCUSSION

The rearing performance of all the parental breeds, foundation crosses and double hybrids is presented in Tables 1, 2 and 3 respectively.

## 3.1 Fecundity (No.)

Significant variation in fecundity was observed in all the parents and their hybrids. Fecundity is a hereditary character and its expression is in direct correlation with several physiological and ecological factors. From a breeding point of view, fecundity plays a vital role because it denotes the efficacy of any silkworm line to be selected as a parental breed for grainage purposes. Among the parental breeds, B1 recorded significantly highest fecundity of 524.00 followed by CSR2 (512.00). In foundation crosses, B1 × B2 recorded significantly highest fecundity (553.67) followed by FC2 (541.67). In double hybrids, (B1 × B2) × FC1 exhibited significantly highest fecundity of 614.33 surpassing the control FC2 × FC1 (597.33).

**Table 1. Performance of oval and dumbbell parental breeds for reproductive and growth traits**

| Breeds | Fecundity (No.) | Hatching (%) | Dead eggs (%) | Larval mortality (%) | Larval duration (h) | Fifth instar larval weight (g/10 larvae) | Pupal weight (g) | ERR (%) | Pupation rate (%) | CSI (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 524.00$^{a}$ | 98.67$^{a}$ | 1.33$^{h}$ | 3.09$^{f}$ | 165.48$^{cd}$ | 31.27$^{a}$ | 1.31$^{a}$ | 96.91$^{a}$ | 96.84$^{a}$ | 93.84$^{a}$ |
| B2 | 477.00$^{d}$ | 95.47$^{abcd}$ | 4.53$^{d}$ | 3.87$^{d}$ | 178.54$^{a}$ | 29.87$^{abc}$ | 1.07$^{cd}$ | 96.13$^{a}$ | 96.06$^{a}$ | 92.34$^{a}$ |
| B4 | 509.00$^{ab}$ | 97.84$^{ab}$ | 2.16$^{g}$ | 3.57$^{e}$ | 164.28$^{cd}$ | 30.11$^{abc}$ | 1.28$^{a}$ | 96.43$^{a}$ | 96.64$^{a}$ | 93.18$^{a}$ |
| CSR2 | 512.00$^{ab}$ | 97.44$^{ab}$ | 2.56$^{f}$ | 3.01$^{f}$ | 160.21$^{d}$ | 31.47$^{a}$ | 1.26$^{a}$ | 96.99$^{a}$ | 96.68$^{a}$ | 93.76$^{a}$ |
| CSR27 | 501.00$^{abc}$ | 95.97$^{abc}$ | 4.03$^{e}$ | 4.02$^{cd}$ | 176.87$^{a}$ | 30.67$^{ab}$ | 1.18$^{b}$ | 95.98$^{a}$ | 95.22$^{a}$ | 91.39$^{a}$ |
| B6 | 471.00$^{d}$ | 92.19$^{d}$ | 7.81$^{a}$ | 6.03$^{a}$ | 177.57$^{a}$ | 27.65$^{d}$ | 1.02$^{d}$ | 93.97$^{a}$ | 92.33$^{a}$ | 86.76$^{c}$ |
| B8 | 464.00$^{d}$ | 93.21$^{cd}$ | 6.79$^{b}$ | 5.98$^{a}$ | 180.67$^{a}$ | 28.67$^{cd}$ | 1.01$^{d}$ | 94.02$^{a}$ | 93.29$^{a}$ | 87.71$^{bc}$ |
| CSR6 | 492.00$^{bcd}$ | 94.61$^{bcd}$ | 5.39$^{c}$ | 4.17$^{c}$ | 175.34$^{ab}$ | 29.34$^{bcd}$ | 1.05$^{cd}$ | 95.83$^{a}$ | 94.09$^{a}$ | 90.16$^{abc}$ |
| CSR26 | 506.00$^{ab}$ | 96.01$^{abc}$ | 3.99$^{e}$ | 4.47$^{b}$ | 170.29$^{bc}$ | 29.47$^{bc}$ | 1.10$^{c}$ | 95.53$^{a}$ | 94.98$^{a}$ | 90.73$^{ab}$ |
| F Test | * | * | * | * | * | * | * | NA | NA | * |
| S.Em ± | 8.509 | 1.009 | 0.056 | 0.056 | 2.052 | 0.536 | 0.021 | 1.667 | 1.642 | 1.103 |
| CD@5% | 25.476 | 3.021 | 0.168 | 0.168 | 6.143 | 1.606 | 0.062 | NA | NA | 3.302 |
| CV (%) | 2.977 | 1.826 | 2.265 | 2.287 | 2.064 | 3.114 | 3.126 | 3.682 | 2.99 | 2.097 |

**-Significant at 5 %; NA - Not analysed; Figures with same superscript are statistically on par*

**Table 2. Performance of oval and dumbbell foundation crosses for reproductive and growth traits**

| Foundation crosses | Fecundity (No.) | Hatching (%) | Dead eggs (%) | Larval mortality (%) | Larval duration (h) | Fifth instar larval weight (g/10 larvae) | Pupal weight (g) | ERR (%) | Pupation rate (%) | CSI (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| B1 × B2 | 553.67$^{a}$ | 95.20$^{ab}$ | 4.80$^{c}$ | 2.67$^{e}$ | 159.21$^{c}$ | 34.71$^{a}$ | 1.53$^{a}$ | 97.33$^{a}$ | 94.98$^{abc}$ | 93.27$^{a}$ |
| B1 × B4 | 537.33$^{ab}$ | 96.10$^{ab}$ | 3.90$^{d}$ | 2.79$^{de}$ | 165.18$^{b}$ | 35.39$^{a}$ | 1.40$^{b}$ | 97.21$^{a}$ | 95.29$^{ab}$ | 92.44$^{ab}$ |
| B2 × B4 | 522.67$^{b}$ | 92.34$^{b}$ | 7.66$^{a}$ | 3.89$^{b}$ | 174.11$^{a}$ | 31.41$^{b}$ | 1.09$^{d}$ | 96.11$^{a}$ | 93.22$^{bc}$ | 89.59$^{ab}$ |
| FC2$^{\$}$ | 541.67$^{ab}$ | 97.60$^{a}$ | 2.40$^{f}$ | 3.17$^{c}$ | 157.21$^{c}$ | 35.64$^{a}$ | 1.41$^{b}$ | 96.83$^{a}$ | 96.33$^{a}$ | 92.63$^{ab}$ |
| B6 × B8 | 497.00$^{c}$ | 93.98$^{ab}$ | 6.02$^{b}$ | 4.42$^{a}$ | 167.67$^{b}$ | 30.62$^{b}$ | 1.07$^{d}$ | 95.58$^{a}$ | 92.33$^{c}$ | 88.24$^{b}$ |
| FC1$^{\$}$ | 529.67$^{b}$ | 96.30$^{ab}$ | 3.07$^{e}$ | 2.86$^{d}$ | 165.56$^{b}$ | 31.96$^{b}$ | 1.28$^{c}$ | 97.14$^{a}$ | 94.29$^{abc}$ | 91.59$^{ab}$ |
| F Test | * | * | * | * | * | * | * | NA | NA | NA |
| S.Em ± | 6.523 | 1.324 | 0.068 | 0.044 | 1.267 | 0.590 | 0.014 | 1.684 | 0.830 | 1.398 |
| CD@5% | 20.320 | 0.056 | 0.211 | 0.139 | 3.948 | 1.839 | 0.043 | NA | NA | NA |
| CV (%) | 2.130 | 2.407 | 2.529 | 2.334 | 1.332 | 3.072 | 1.827 | 3.016 | 1.524 | 2.653 |

**- Significant at 5 %; NA - Not analysed; Figures with same superscript are statistically on par*
*$ -FC1 and FC2 are commercial foundation crosses*

**Table 3. Performance of double hybrids for reproductive and growth traits**

| Double hybrids | Fecundity (No.) | Hatching (%) | Dead eggs (%) | Larval mortality (%) | Larval duration (h) | Fifth instar larval weight (g/10 larvae) | Pupal weight (g) | ERR (%) | Pupation rate (%) | CSI (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| (B1 × B2) × (B6 × B8) | 522.00$^{cd}$ | 94.81$^{b}$ | 2.77$^{f}$ | 3.32$^{d}$ | 148.81$^{bc}$ | 43.44$^{de}$ | 1.92$^{b}$ | 96.68$^{a}$ | 97.29$^{a}$ | 94.05$^{a}$ |
| (B1 × B2) × (FC1)$ | 614.33$^{a}$ | 97.23$^{a}$ | 4.92$^{d}$ | 1.41$^{g}$ | 142.11$^{cd}$ | 48.33$^{b}$ | 2.06$^{a}$ | 98.59$^{a}$ | 98.78$^{a}$ | 97.24$^{a}$ |
| (B1 × B4) × (B6 × B8) | 501.33$^{d}$ | 95.08$^{ab}$ | 2.52$^{f}$ | 4.09$^{b}$ | 140.81$^{cd}$ | 43.8$^{de}$ | 1.78$^{c}$ | 95.91$^{a}$ | 97.05$^{a}$ | 93.08$^{a}$ |
| (B1 × B4) × (FC1)$ | 568.00$^{b}$ | 97.42$^{a}$ | 6.48$^{a}$ | 1.59$^{g}$ | 140.26$^{cd}$ | 47.10$^{b}$ | 2.03$^{a}$ | 98.41$^{a}$ | 98.64$^{a}$ | 97.21$^{a}$ |
| (B2 × B4) × (B6 × B8) | 498.67$^{d}$ | 93.52$^{b}$ | 5.52$^{b}$ | 4.73$^{a}$ | 134.92$^{d}$ | 42.55$^{e}$ | 1.85$^{bc}$ | 95.27$^{a}$ | 97.01$^{a}$ | 92.42$^{a}$ |
| (B2 × B4) × (FC1)$ | 528.33$^{c}$ | 94.48$^{b}$ | 3.18$^{e}$ | 3.61$^{c}$ | 141.11$^{cd}$ | 46.44$^{bc}$ | 2.03$^{a}$ | 96.39$^{a}$ | 98.41$^{a}$ | 94.85$^{a}$ |
| (FC2)$ × (B6 × B8) | 541.33$^{c}$ | 96.65$^{ab}$ | 3.35$^{e}$ | 3.13$^{e}$ | 156.11$^{b}$ | 44.65$^{cd}$ | 1.92$^{b}$ | 96.87$^{a}$ | 97.65$^{a}$ | 95.59$^{a}$ |
| FC2$ × FC1$ | 597.33$^{a}$ | 96.82$^{ab}$ | 5.19$^{c}$ | 1.91$^{f}$ | 166.81$^{a}$ | 51.96$^{a}$ | 2.02$^{a}$ | 98.09$^{a}$ | 98.94$^{a}$ | 97.05$^{a}$ |
| F Test | * | * | * | * | * | * | * | NA | NA | NA |
| S.Em ± | 7.712 | 1.551 | 0.086 | 0.055 | 2.624 | 0.601 | 0.03 | 1.205 | 1.128 | 1.635 |
| CD@5% | 23.319 | 0.431 | 0.261 | 0.166 | 7.934 | 1.819 | 0.091 | NA | NA | NA |
| CV (%) | 2.444 | 2.806 | 3.527 | 3.197 | 3.105 | 2.263 | 2.678 | 2.150 | 1.994 | 2.975 |

**- Significant at 5 %; NA - Not analysed; Figures with same superscript are statistically on par; $ -FC1 and FC2 are commercial foundation crosses used in combination with the new bivoltine silkworm foundation crosses*

Present results are consistent with earlier studies. [8] recorded a fecundity of 540.00 in CSR2 × CSR4 and Jayachamaraja recorded highest fecundity (625.00), surpassing Krishnaraja (620.00). Variation in fecundity was found in different breeds and hybrids, which resulted in fluctuation of egg number per brood. Fecundity mainly depends upon the genotype of mother moth and environmental conditions at the time of oviposition [9].

### 3.2 Hatching Percentage (%)

Significant variation in hatching percentage was observed in all the parents and their hybrids. Hatching percentage serves as a key commercial trait for hybrid validation, reflecting egg viability. Insect survival and development are subject to ecological conditions and genetic factors, influencing their biological activities [10]. Among parental breeds, B1 recorded significantly highest hatching percentage of 98.67 per cent followed by B4 (97.44%). In foundation crosses, FC2 recorded significantly highest hatching percentage (97.60%) followed by FC1 (96.30%). In double hybrids, (B1 × B4) × FC1 exhibited significantly highest hatching percentage of 97.42 per cent followed by (B1 × B2) × FC1 (97.23%).

These results are in conformity with earlier studies by [11] who recorded highest hatching percentage in CSR50 × APS5 (97.25%) compared to FC2 (96.27%). Higher hatching percentages of some breeds and hybrids could be attributed to their genetic traits favouring better egg viability, embryonic development and the physiological condition of the female moth [12]. High hatching percentage observed in bivoltine breeds also reflects the high value for number of eggs hatched, number of warms brushed and brushing percentage which are important characters of quality silkworm seed and breed.

### 3.3 Dead eggs Percentage (%)

Significant variation in dead eggs was observed in all the parents and their hybrids. Dead eggs percentage indicated the proportion of nonviable eggs, reflecting egg quality and potential issues with female moth health, mating or rearing conditions. Among parental breeds, B1 recorded significantly least dead egg percentage of 1.33 per cent compared to control followed by B4 (2.16%). In foundation crosses, FC2 recorded significantly least dead egg percentage (2.40%) followed by FC1 (3.70%). In double hybrids, (B1 × B4) × FC1 exhibited significantly least dead egg percentage of 2.52 per cent followed by (B1 × B2) × FC1 (2.77%).

The current findings corroborate with prior research. [13] reported least dead egg percentage in foundation crosses in HL1 × HL7 (1.32%) compared to the control CSR2 × CSR4 (2.78%). Considerable amount of reduction in dead egg percentage was noticed in double hybrids compared to the pure races and foundation crosses [14].

### 3.4 Larval Mortality (%)

Significant variation in larval mortality was observed in all the parents and their hybrids. Among parental breeds, CSR2 recorded significantly least larval mortality of 3.01 per cent followed by B1 (3.09%). In foundation crosses, B1 × B2 recorded significantly least larval mortality (2.67%) compared to CSR breeds, followed by B1 × B4 (2.79%). In double hybrids, (B1 × B4) × FC1 exhibited significantly least larval mortality of 1.41 per cent followed by (B1 × B2) × FC1 (1.59%) both surpassing FC2 × FC1.

Comparable findings have been reported in earlier studies. [15] reported least mortality in O323 × H155 (0.70%), followed by J325 × O323 (1.05%). Further, [16] reported least mortality rate in CSR26 × CSR27 (3.67%). The present findings about larval mortality underline the influence of environmental conditions and genetic traits on silkworm survival rates. Hybrids with lower mortality are considered better performers because they are less affected by the external factors.

### 3.5 Fifth Instar Larval Duration (h)

Fifth instar larval duration was calculated from the first day of the fifth instar until 50 per cent spinning. Significant variation in fifth instar larval duration was observed in parents and their hybrids. Larval duration is considered as an important attribute of economic value in sericulture as the reduction in larval duration would not only help in minimizing the quantum of the food consumption by the insect but also in completion of larval period in desirable time period besides minimizing the labour requirement [17]. Among parental breeds, CSR2 recorded significantly least fifth instar larval duration of 160.21 h followed by B4 (164.28 h). In foundation crosses, FC2 recorded significantly least fifth instar larval duration (157.21h), followed by B1 × B2 (159.21 h). In double hybrids, (B2 × B4) × (B6 × B8) exhibited significantly least fifth instar larval duration of 134.92 h, followed by (B1 × B4) × FC1 (140.26 h).

In a recent study, [18] reported highest fifth instar larval duration in FC1 × FC2 (149.52 h), followed by PO3 × ND5 (149.28 h) which are shorter compared to the present findings. In *B. mori*, the larval duration, while genetically determined, is also influenced by various factors, including macro and micro-environmental conditions, as well as the rearing skills [19]. This diversity can be attributed to the hybrids responsiveness to rearing practices and *in-vitro* conditions maintained during the rearing period.

### 3.6 Fifth Instar Larval Weight (g/10 larave)

Significant variation in fifth instar larval weight was observed in parents and their hybrids. Fifth instar larval weight is one of the important parameters that determines not only the health of the larvae, but also the quality of the cocoons spun [20]. Among parental breeds, CSR2 recorded significantly highest fifth instar larval weight of 31.47 g followed by B1 (31.27 g). In foundation crosses, FC2 recorded significantly highest fifth instar larval weight (35.64 g), followed by B1 × B4 (35.39 g). In double hybrids, FC2 × FC1 exhibited significantly highest fifth instar larval weight of 51.96 g, followed by (B1 × B2) × FC1 (48.33 g).

The observed variation in larval weight among hybrids aligns with earlier studies. [21] reported highest fifth instar larval weight in FC2 × FC1 (44.04 g/10 larvae) followed by CSR16 × CSR17 (43.91 g/ 10 larvae). The differences in grownup larval weight among the breeds and hybrids studied could be attributed to the racial character, differences in degree of assimilation and the quality and quantity of feed consumed by the larvae which has a direct bearing on the growth and development of larvae. This superiority can be attributed to their good genetic variability, potentially favourable combinations of parental traits and responsive growth patterns, which collectively contribute to enhanced larval growth and weight gain [12].

### 3.7 Pupal Weight (g)

Significant variation in pupal weight was observed in all the parents and their hybrids. The difference between cocoon and shell weight is the weight of the pupa and is considered important with respect to commercial traits evaluated for productivity in sericulture [22]. Among parental breeds, B1 recorded significantly highest pupal weight of 1.31 g followed by B4 (1.28 g). In foundation crosses, B1 × B2 recorded significantly highest pupal weight (1.53 g), followed by FC2 (1.41 g). In double hybrids, FC2 × FC1 exhibited significantly highest pupal weight of 2.06 g, followed by (B1 × B2) × FC1 (2.03 g).[23] noted the maximum pupal weight in $F_1$'s of the same breeds used in the present study *viz.,* B1 × B4 (1.46 g) followed by B1 × B8 and B4 × B1 (1.44 g each). [24] documented highest pupal weight in the hybrid B1 × CSR4 (1.54 g), followed by B4 × CSR4 (1.52 g). The pupal weight of *B. mori* has been noticed to be influenced by the variation in the level of secreted hormones and genotype variation. Higher pupal weight attained by some breeds and hybrids indicates their better feed consumption and good larval growth during the larval periods which could also be attributed to better larval growth period and reduced meltage during pupal development [25].

### 3.8 ERR (%)

No significant difference was observed for this trait among parents, foundation crosses and double hybrids. A higher value of ERR is indicative of higher survival and thereby silk productivity. It is important to study the survival probability of silkworm breeds and hybrids. Among parental breeds, CSR2 recorded highest ERR of 96.99 per cent followed by B1 (96.91) and all other pure breeds were on par statistically. In foundation crosses, B1 × B2 recorded highest ERR (97.33%), followed by FC2 (97.21%) and other FCs were on par with each other. In double hybrids, (B1 × B4) × FC1 exhibited highest ERR of 98.59 per cent followed by (B1 × B2) × FC1 (98.41%) and other double hybrids were on par statistically.

The present outcomes are supported by [16] who reported highest ERR (96.33%) in CSR26 × CSR27. In a thermotolerant double hybrid TT21 × TT56, highest ERR of 89.20 per cent was observed, surpassing FC1 × FC2 (82.24%) [26]. Survival percentage is directly related with the cocoon yield and hence more weight age has to be given for survival while evaluating the FCs of the hybrids and identifying them for exploitation [27]. The insignificant variation for larval survival can be attributed to uniform rearing condition and non-occurrence of disease. In the preset study, equally superior performance of some hybrids concerning ERR could be attributed to optimum rearing conditions, selection of parent lines with desirable traits, resulting in hybrids that inherit advantageous genetic characteristics, efficient growth patterns and potentially optimized metabolic processes.

### 3.9 Pupation Rate (%)

No significant difference was observed for pupation among parents, foundation crosses and double hybrids. Pupation rate signifies the number of cocoons containing live pupae, holds paramount importance as it directly impacts cocoon yield and the quality of silk production [11]. It is particularly crucial for seed cocoon crops, as far as farmers are concerned because it fetches better price for their cocoons. To determine the variability of a breed/hybrid it is one of the important economic characters. Among parental breeds, B1 recorded highest pupation rate of 96.84 per cent followed by CSR 2 (96.68%) and all other pure breeds were on par statistically. In foundation crosses, FC2 recorded highest pupation rate (96.33%), followed by B1 × B4 (95.29%) and all other FCs were on par statistically. In double hybrids, FC2 × FC1 exhibited highest pupation rate of 98.94 per cent, followed by (B1 × B2) × FC1 (98.78%) and all other double hybrids were on par statistically.

The observations follow the findings of [9] who observed highest pupation rate of 95.20 per cent in Jayachamaraja surpassing Krishnaraja (95.10%). There is significant improvement in the rate of pupation in foundation crosses and double hybrids over parental breeds due to positive heterosis. Good pupation percentage is a positive sign for cocoon reeling performance as well as seed production. These are generally influenced by rearing environment and other abiotic factors. The genetic and environmental interaction is more reflected in this character [28]. The insignificant variation in pupation rate could be attributed to the controlled rearing environment provided, which optimized temperature and humidity conditions, fostering favourable pupal development and survival for each hybrid combination.

### 3.10 Cumulative Survival Index (%)

No significant difference was observed for pupation among parents, foundation crosses and double hybrids. CSI is determined by combining effective rate of rearing and pupation rates, serves as a comprehensive measure of the overall survival ability of breeds and hybrids across their entire lifecycle, from the larval stage to moth emergence. ERR and pupation rate being of paramount importance in deciding the survivability of silkworm breeds and hybrids, the cumulative survival indices were calculated to know the overall survival ability utilizing those values for all the breeds and hybrids in the study. Among nine parental breeds, B1 recorded significantly highest pupation rate of 93.84 % followed by CSR 2 (93.76). In foundation crosses, B1 × B4 recorded highest pupation rate (93.27%), followed by FC2 (92.63%). Among eight double hybrids, (B1 × B2) × FC1 exhibited significantly highest pupation rate of 97.24%, followed by (B1 × B4) × FC1 (97.21%). These findings align with the outcomes reported by [29] who observed a 100 per cent CSI in Pure Mysore, followed by CSR2 (91.11%) and B4 (90.67%).

## 4. CONCLUSION

In summary, the parental breeds demonstrated their purity and consistency, maintaining the original breed characteristics. Both foundation crosses and double hybrids exhibited a higher level of hybrid vigor surpassing the parental breeds. Double hybrids have shown greater variability, followed closely by foundation crosses. Parental breeds B1, CSR2 and B4 consistently performed well across all evaluated traits, suggesting their suitability as oval parents in new hybrids preparation. Similarly, foundation crosses B1 × B2, FC2 and B1 × B4 also demonstrated positive traits across all the traits. Double hybrids (B1 × B2) × (FC1) and (B1 × B4) × (FC1) performed on par with control (FC2 × FC1) and showed promising performance. Consequently, these new double hybrids can be further evaluated in field trials and can be considered viable alternatives to the FC2 × FC1 hybrid.

## ACKNOWLEDGEMENT

The authors are thankful to Central Sericultural Research and Training Institute, Mysore for timely supply of parental bivoltine silkworm eggs to conduct the experiments.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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