Journal of Advances in Biology & Biotechnology

Volume 27, Issue 8, Page 955-963, 2024; Article no.JABB.120611
ISSN: 2394-1081

Performance of New Bivoltine Silkworm Breeds and Hybrids for Economic Traits

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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. Authors MG and KCN Planned the study and arranged for the silkworm breeds. Authors Chikkalingaiah, JS and SR, supported in designing the breeding schemes. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i81216

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/120611

Original Research Article

Received: 26/05/2024
Accepted: 29/07/2024
Published: 03/08/2024

ABSTRACT

An assessment of new bivoltine races and their hybrids was conducted to identify the best-performing hybrid. Five thermotolerant pure breeds (B1, B2, B4, B6 and B8) and four popular CSR breeds (CSR2, CSR27, CSR6, CSR26) were used to develop six foundation crosses and eight

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Cite as: *D, Thrilekha, Manjunatha Gowda, Chethan Kumar D.S, Chikkalingaiah, J. Seetharamulu, K. C. Narayanaswamy, and S. Ramesh. 2024. "Performance of New Bivoltine Silkworm Breeds and Hybrids for Economic Traits". Journal of Advances in Biology & Biotechnology 27 (8):955-63. https://doi.org/10.9734/jabb/2024/v27i81216.*

double hybrids. These breeds and hybrids were evaluated based on eight important economic traits. Among the parental races, B4 excelled in four traits viz., single cocoon weight (1.69 g), cocoon yield/10,000 larvae (16.42 kg), filament length (1347.14 m) and filament weight (0.31 g). CSR27 recorded the highest shell weight (0.34 g), shell ratio (22.18%) and denier (2.49). In foundation crosses, B1 × B4 had the highest single cocoon weight (1.81 g), cocoon yield/10,000 larvae (17.98 kg), filament length (1319.06 m) and filament weight (0.33 g). FC2 excelled in shell weight (0.38 g), shell ratio (22.33%) and denier (2.45). Among double hybrids, (B1 × B4) × (FC1) recorded the highest single cocoon weight (2.67 g), cocoon yield/10,000 larvae (25.87 kg), filament length (1434.29 m) and denier (2.85). FC2 × FC1 performed best in shell weight (0.38 g), shell ratio (22.33%) and filament weight (0.47 g). Thus, new breeds B1 and B4, foundation crosses B1 × B4 and B1 × B2 and double hybrids (B1 × B4) × (FC1) show promise for commercial exploitation after multi-location trials, offering alternatives to established CSR breeds and hybrids.

Keywords: Bombyx mori; bivoltine silkworm; double hybrids; commercial traits; foundation crosses; parental breeds.

1. INTRODUCTION

Mulberry silkworm, *Bombyx mori* L., is an economically important insect commercially exploited for silk production. In recent years, India has witnessed a notable shift towards rearing bivoltine silkworms, aimed at enhancing silk quality to meet international standards. This transition is driven by the increasing demand for import substitute raw silk, underscoring the significance of bivoltine silkworm breeds and hybrids in the sericulture sector [1]. Despite efforts by sericultural authorities, the widespread adoption of bivoltine silkworm breeds/hybrids in India has been hindered by several factors. The primary constraints are instability in cocoon yield and limited suitability to diverse climatic conditions. Currently, the Indian sericulture landscape predominantly relies on a few bivoltine hybrids tailored for optimal conditions during favourable seasons. To address these limitations and promote sustainable sericulture practices, there is a pressing need for the development of new silkworm breeds and hybrids conducive to commercial exploitation.

However, the process of breeding new silkworm varieties must navigate the pitfalls associated with inbreeding, which can lead to decreased genetic variance and undesirable traits in subsequent generations. While single hybrids derived from genetically distant parents exhibit hybrid vigour and productivity, double hybrids, combining characteristics from four diverse parental breeds, offer greater stability and financial returns to farmers. This stability is attributed to the polygenic expression of economic traits within the population, ensuring resilience under sub-optimal conditions. Despite the known advantages of double hybrids, the selection of suitable hybrid combinations for commercial exploitation is crucial for success. Therefore, this study aims to identify and evaluate promising bivoltine breeds and hybrids for their performance and suitability for commercialization.

2. MATERIALS AND METHODS

The present study was conducted at the Department of Sericulture, University of Agricultural Sciences, GKVK, Bengaluru during 2022-2023. Five thermotolerant bivoltine silkworm breeds *viz.,* B1, B2, B4 (oval cocoon spinning breeds) B6 and B8 (pea nut cocoon spinning breeds) procured from CSRTI, Mysore and four productive CSR parental races (CSR2, CSR27, CSR6 and CSR26) obtained from NSSO 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.

Foundation crosses were developed using oval × oval and dumbbell × dumbbell breeds. These foundation crosses (FCs) were employed in double hybrid preparations (oval × oval) × (dumbbell × dumbbell). Deflossed cocoons were cut at one end to remove the pupae for sex determination, based on morphological characteristics such as markings and size. Pupae from all the pure races and foundation crosses were segregated by sex and preserved in separate trays. To induce simultaneous moth emergence, male pupae were refrigerated 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 disease free layings of all the pure lines and hybrids were incubated under optimum environmental conditions of 25±1°C temperature and 75±5% relative humidity until 8^{th} day, followed by black boxing to achieve uniform hatching. Newly hatched larvae were brushed with freshly chopped mulberry leaves. Silkworm rearing was conducted using a suitable rearing method [2]. During rearing, three feedings with fresh V-1 mulberry leaves were provided to all the worms until the onset of spinning. All the parents and hybrids were reared following a completely randomized design with three replications each, maintaining 200 larvae in each replication after the 3^{rd} moult. At the end of the 5^{th} instar, the spinning larvae were collected manually and transferred to bamboo mountages. The cocoons were harvested on the 6^{th} or 7^{th} day of mounting and assessed the following day for commercial parameters *viz.,* Cocoon yield per 10,000 larvae by number and by weight (kg), single cocoon weight (g), single shell weight (g), shell ratio (%) Filament length (m), Filament weight (g) and denier. Using a completely randomized design, the mean data gathered from two rearings was analysed using OP STAT software. Duncan's Multiple Range Test (DMRT) was used to compare the mean values of the study [3].

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 Single Cocoon Weight (g)

Significant variation in single cocoon weight was observed in all the pure breeds and their hybrids. Cocoon weight is closely correlated with cocoon yield, significantly impacting the financial return for farmers. It is an important trait, used to determine approximate amount of raw silk that can be reeled. Among the parental breeds, B4 recorded significantly highest cocoon weight of 1.69 g followed by CSR27 (1.67 g). In foundation crosses, B1× B4 recorded significantly highest cocoon weight (1.81 g) followed by FC2 (1.76 g). In double hybrids, (B1 × B4) × FC1 exhibited significantly highest cocoon weight of 2.67 g followed by (B1 × B2) × FC1 (2.62 g) surpassing the control FC2 × FC1 (2.61 g).

These results are comparable with earlier investigations. Shabir et al [4] recorded highest single cocoon weight in SK28 × SBNP1 (1.61 g) followed by NB4D2 × SH6 (1.59 g). Gowda *et al*. (2013) recorded a cocoon weight of 2.04 g for the double hybrid (CSR2 × CSR27) × (CSR6 × CSR26). The superior cocoon weight of certain parental breeds and hybrids likely indicates differences in nutrient utilization during the fifth instar and their genetic potential.

3.2 Cocoon Yield (number/10,000 larvae)

There is no significant difference observed for cocoon yield by number among parents, foundation crosses and double hybrids. Among the parental breeds, CSR2 recorded highest cocoon yield of 9655.43/10,000 larvae followed by B1 (9654.91/10,000 larvae). In foundation crosses, B1× B2 recorded highest cocoon yield (9799.14/10,000 larvae) followed by B1× B4 (9766.33/10,000 larvae). In double hybrids, (B1 × B2) × FC1 exhibited highest cocoon yield of 9844.59 surpassing the control FC2 × FC1 (9840.59/10,000 larvae).

The current findings align with previous studies by Gowda et al [5] who reported the highest cocoon yield in (CSR27 × CSR17) × (D13 × CSR26) (9581.00/10,000 larvae) followed by FC2 × FC1 (9155.00/10,000 larvae). Dayananda et al [6] reported highest cocoon yield in CSR4 × CSR2 (9840.00/10,000 larvae) over control FC2 × FC1 (9744.00/10,000 larvae). In the present study, all the parents and hybrids showed comparably better performance in cocoon yield by number per 10,000 larvae. This improved performance can be attributed to optimal rearing conditions and the availability of nutritious mulberry leaves, leading to better survivability and yield.

Table 1. Performance of parental breeds for economic traits

Breeds	Single Cocoon Weight (g)	Cocoon yield by Number (Per 10,000 larvae)	Cocoon Yield by Weight (Per 10,000 larvae)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Filament Weight (g)	Denier
B1	1.59^{b}	9654.91^{a}	14.54^{b}	0.30^{abcd}	21.42^{ab}	1287.34^{bc}	0.26^{ab}	2.34^{b}
B2	1.37^{cd}	9604.13^{a}	12.94^{d}	0.27^{bcd}	19.23^{e}	1194.61^{de}	0.24^{ab}	2.09^{fg}
B4	1.69^{a}	9633.99^{a}	16.42^{a}	0.33^{ab}	22.14^{a}	1347.14^{a}	0.29^{a}	2.49^{a}
CSR2	1.57^{b}	9655.43^{a}	14.29^{bc}	0.31^{abc}	21.16^{bc}	1267.09^{bc}	0.27^{ab}	2.28^{bc}
CSR27	1.67^{a}	9566.53^{a}	16.10^{a}	0.34^{a}	22.18^{a}	1320.14^{ab}	0.31^{a}	2.46^{a}
B6	1.30^{e}	9302.97^{a}	12.49^{de}	0.25^{cd}	19.10^{e}	1147.34^{e}	0.23^{ab}	2.02^{g}
B8	1.29^{e}	9422.02^{a}	12.06^{e}	0.24^{d}	18.60^{e}	1021.39^{f}	0.20^{b}	2.14^{ef}
CSR6	1.34^{de}	9544.98^{a}	12.54^{de}	0.29^{abcd}	19.52^{de}	1197.64^{de}	0.27^{ab}	2.19^{de}
CSR26	1.42^{c}	9533.83^{a}	13.69^{c}	0.28^{abcd}	20.35^{cd}	1245.87^{cd}	0.26^{ab}	2.23^{cd}
F Test	*	NA	*	*	*	*	*	*
S.Em ±	**0.017**	**145.843**	**0.232**	**0.019**	**0.295**	**17.633**	**0.024**	**0.025**
CD@5%	**0.051**	**NA**	**0.695**	**0.057**	**0.883**	**52.795**	**0.073**	**0.076**
CV (%)	**2.019**	**2.646**	**2.893**	**1.064**	**2.502**	**2.492**	**1.449**	**1.952**

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

Table 2. Performance of foundation crosses for economic traits

Foundation Crosses	Single Cocoon Weight (g)	Cocoon Yield by Number (Per 10,000 larvae)	Cocoon Yield by Weight (Per 10,000 larvae)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Filament Weight (g)	Denier
B1 × B2	1.63^{a}	9799.14^{a}	15.39^{c}	0.33^{ab}	21.63^{b}	1299.29^{a}	0.29^{ab}	2.39^{ab}
B1 × B4	1.81^{a}	9766.33^{a}	17.98^{a}	0.35^{ab}	21.98^{ab}	1319.06^{a}	0.30^{ab}	2.45^{a}
B2 × B4	1.35^{b}	9622.11^{a}	13.12^{d}	0.30^{ab}	19.37^{d}	1193.23^{b}	0.26^{ab}	2.17^{c}
FC2$	1.76^{a}	9622.83^{a}	16.97^{b}	0.38^{a}	22.33^{a}	1301.16^{a}	0.33^{a}	2.42^{ab}
B6 × B8	1.30^{b}	9544.58^{a}	11.97^{e}	0.27^{b}	20.27^{c}	1159.58^{b}	0.23^{b}	2.24^{bc}
FC1$	1.41^{b}	9711.21^{a}	13.67^{d}	0.31^{ab}	21.48^{b}	1278.80^{a}	0.27^{ab}	2.40^{ab}
F Test	*	NA	*	*	*	*	*	*
S.Em ±	**0.058**	**167.622**	**0.241**	**0.03**	**0.207**	**21.008**	**0.023**	**0.061**
CD@5%	**0.182**	**NA**	**0.749**	**0.095**	**0.644**	**65.449**	**0.072**	**0.189**
CV (%)	**1.424**	**3.00**	**2.805**	**1.624**	**1.690**	**2.891**	**1.331**	**1.195**

**- 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 economic traits

Double Hybrids	Single Cocoon Weight (g)	Cocoon Yield by Number (Per 10,000 larvae)	Cocoon Yield by Weight (Per 10,000 larvae)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Filament Weight (g)	Denier
B1 × B2) × (B6 × B8)	2.45^{abc}	9677.87^{a}	23.64^{c}	0.48^{bc}	20.08^{b}	1288.87^{b}	0.40^{bcd}	2.78^{b}
(B1 × B2) × (FC1) $	2.62^{a}	9844.59^{a}	25.67^{a}	0.57^{abc}	22.86^{a}	1422.69^{a}	0.44^{abc}	2.84^{ab}
(B1 × B4() × (B6 × B8)	2.47^{abc}	9566.91^{a}	23.97^{bc}	0.54^{abc}	21.40^{b}	1330.51^{b}	0.43^{abc}	2.75^{bc}
(B1 × B4) × (FC1) $	2.67^{a}	9822.09^{a}	25.87^{a}	0.58^{ab}	23.13^{a}	1434.29^{a}	0.45^{ab}	2.85^{ab}
(B2 × B4) × (B6 × B8)	2.29^{c}	9599.27^{a}	21.64^{d}	0.47^{c}	20.96^{b}	1272.38^{b}	0.36^{d}	2.55^{d}
(B2 × B4) × (FC1) $	2.34^{bc}	9611.39^{a}	22.94^{cd}	0.52^{abc}	21.23^{b}	1403.04^{a}	0.42^{abcd}	2.65^{cd}
(FC2) $ × (B6 × B8)	2.57^{ab}	9677.87^{a}	24.21^{bc}	0.50^{abc}	22.83^{a}	1307.42^{b}	0.38^{cd}	2.76^{bc}
FC2 $ × FC1 $	2.61^{a}	9840.59^{a}	25.19^{ab}	0.59^{a}	23.54^{a}	1422.01^{a}	0.47^{a}	2.95^{a}
F Test	*	NA	*	*	*	*	*	*
S.Em ±	**0.076**	**99.342**	**0.398**	**0.032**	**0.389**	**19.960**	**0.021**	**0.036**
CD@5%	**0.229**	**NA**	**1.203**	**0.096**	**1.177**	**60.354**	**0.062**	**0.108**
CV (%)	**1.441**	**1.774**	**2.854**	**1.316**	**3.064**	**2.542**	**0.96**	**2.244**

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

$ -FC1 and FC2 are commercial foundation crosses used as combination with the new bivoltine silkworm foundation crosses

3.3 Cocoon Yield (kg/10,000 larvae)

Significant variation in cocoon yield by weight was observed in all the parental breeds and their hybrids. Cocoon yield by weight shows quantum of cocoons obtained after [7]. Among the parental breeds, B4 recorded significantly highest cocoon yield of 16.42 kg/10,000 larvae followed by CSR27 (16.10 kg/10,000 larvae). In foundation crosses, B1× B4 recorded significantly highest cocoon yield (17.98 kg/10,000 larvae) followed by FC2 (16.97 kg/10,000 larvae). In double hybrids, (B1 × B4) × FC1 exhibited significantly highest cocoon yield of 25.87 kg/10,000 larvae followed by (B1 × B4) × FC1 (25.67 kg/10,000 larvae) surpassing the control FC2 × FC1 (25.19 kg/10,000 larvae).

These findings align with earlier studies by Bindroo et al [8] reported highest cocoon yield in Jayachamaraja (20.47 kg/10,000 larvae) over Krishnaraja (19.97 kg/10,000 larvae). Suresh and Singh [9] and Munemanik et al [10] recorded significantly maximum cocoon yield in the double hybrid (CSR2 × CSR27) × (CSR6 × CSR26) (18.40 and 18.84 kg/10,000 larvae, respectively). The difference in the cocoon yield by weight could be attributed to the potential of the breeds and hybrids to adjust to the prevailing environment. In this study, B4, B1× B4, (B1 × B4) × FC1, (B1 × B2) × FC1 and FC2 × FC1 gave higher cocoon yield by weight, highlighting their dominance over the other hybrids. This is likely due to their improved survival rates, reflecting their genetic potential.

3.4 Shell Weight (g)

Significant variation in shell weight was observed in parental breeds and their hybrids. Shell weight is crucial for determining silk quality and yield. It impacts filament length, weaving efficiency and market value of silk products. Among the parental breeds, CSR27 recorded significantly highest shell weight of 0.34 g followed by B4 (0.33 g). In foundation crosses, FC2 recorded significantly highest shell weight (0.38 g) followed by B1× B4 (0.35 g). In double hybrids, FC2 × FC1 exhibited significantly highest shell weight of 0.59 g followed by (B1 × B4) × FC1 (0.58 g).

Rajalakshmi and Sakthivel [11] reported highest shell weight of 0.383 g in SLD1 × SLD3. Gowda et al [12] and Munemanik et al [10] reported shell weight of double hybrid (CSR2 × CSR27) × (CSR6 × CSR26) (0.47 and 0.38 g, respectively). In this study, CSR27, B4, B1× B4, FC2, (B1 × B4) × FC1, (B1 × B2) × FC1 and FC2 × FC1 gave higher shell weight, highlighting their dominance over the other hybrids.

3.5 Shell Ratio (%)

Significant variation in shell ratio was observed in all the parental breeds and their hybrids. A higher shell ratio indicates a larger proportion of silk, contributing to better silk quality and overall commercial value. Among the parental breeds, CSR27 recorded significantly highest shell ratio of 22.18 per cent followed by B4 (22.14%). In foundation crosses, FC2 recorded significantly highest shell ratio (22.33%) followed by B1 × B4 (21.98%). In double hybrids, FC2 × FC1 exhibited significantly highest shell ratio of 23.54 per cent followed by (B1 × B4) × FC1 (23.13%).

These findings are similar to the findings of Suresh et al [13] who observed the maximum shell ratio in DHP5 (21.80%) over rest of the hybrids. Munemanik et al [10] reported highest shell ratio in bivoltine double hybrid (CSR2 × CSR27) × (CSR6 × CSR26) (21.34%). Variations in shell ratio among different parental breeds and hybrids arise from genetic disparities inherited from parental lines and environmental factors like rearing conditions, temperature, humidity and feeding practices.

3.6 Filament Length (m)

Significant variation in filament length was observed in all the parental breeds and their hybrids. Among the parental breeds, B4 recorded significantly highest filament length of 1347.14 m followed by CSR27 (1320.14 m). In foundation crosses, B1× B4 recorded significantly highest filament length (1319.06 m) followed by FC2 (1301.16). In double hybrids, (B1 × B4) × FC1 exhibited significantly highest filament length of 1434.29 m followed by (B1 × B2) × FC1 (1422.69 m) surpassing the control FC2 × FC1 (1422.01 m).

Bindroo et al [8] reported that Jayachamaraja recorded longest filament length (1180.00 m) surpassing Krishnaraja (1075.00 m), which reflects the variation seen in the present findings. In the present study, B4, CSR27, B1× B4, FC2, (B1 × B4) × FC1 and (B1 × B2) × FC1 showed favourable filament length, establishing their superiority among the evaluated breeds and hybrids. This may be attributed to the more efficient synthesis of required proteins and their conversion to raw silk. Superiority in longer filament lengths also arises from genetic factors, favourable traits enhancing silk fibre synthesis and potentially optimized rearing conditions.

3.7 Filament Weight (g)

Significant variation in filament weight was observed in all the parental breeds and their hybrids. Filament weight is crucial in sericulture as it directly impacts silk yield, quality and economic value. Among the parental breeds, B4 recorded significantly highest filament weight of 0.31 g followed by CSR27 (0.29 g). In foundation crosses, B1 × B4 recorded significantly highest filament weight (0.33 g) followed by FC2 (0.30 g). In double hybrids, FC2 × FC1 exhibited significantly highest filament weight of 0.47 g followed by (B1 × B4) × FC1 (0.45 g).

Present findings are in conformity with earlier studies by Bobade et al [14] who recorded highest filament weight in CSR16 × CSR17 (0.331 g) followed by DHP5 (0.261 g). Chandrakala et al [15] recorded highest filament weight in the hybrid, B1 × CSR4 (0.33 g), followed by B4 × CSR4 (0.31 g) where B1 and B4 breeds were used as parents to develop double hybrids in the present study.

3.8 Denier

Significant variation in denier was observed in all the parental breeds and their hybrids. Denier holds significant importance as it quantifies the thickness and quality of silk fibres. It directly influences the market value, durability and versatility of silk products, guiding production processes and consumer preferences. Among the parental breeds, CSR27 recorded significantly highest denier of 2.49 followed by B4 (2.46). In foundation crosses, FC2 recorded significantly highest denier (2.45) followed by B1× B4 (2.42). In double hybrids, (B1 × B2) × FC1 and (B1 × B4) × FC1 recorded medium sized denier of 2.84 and 2.85 respectively, compared to the thicker denier of FC2 × FC1 (2.95).

Datta and Pershad [1] recorded denier of 2.80 in CSR12 × CSR6. Gowda et al [5] recorded a denier of 2.63 in FC2 × FC1. Sivaprasad et al [16] recorded denier of 2.96 in a new double hybrid (G11 × G19) under suboptimal conditions. Usually, medium-sized denier values are preferred. In the present study, CSR27, B4, FC2, B1× B4, (B1 × B4) × FC1 and (B1 × B2) × FC1 demonstrated medium denier value, suggesting finer and higher quality silk fibres that can be obtained from their cocoons compared to other breeds and hybrids in the study.

4. CONCLUSION

In summary, the result of the present study reveals that, out of different breeds and hybrids studied, two each of parental breeds, foundation crosses and double hybrids exhibited better performance with desirable positive heterosis for most of the economic traits. This superior performance can be attributed to better inherent advancement and shows promise for field testing based on the overall performance and heterosis for each quantitative trait studied. Both foundation crosses and double hybrids exhibited a higher level of hybrid vigour, surpassing the parental breeds. Parental breeds B4, CSR27, and B1 consistently excelled across all evaluated traits, indicating their suitability as oval parents in hybrid preparation. Similarly, foundation crosses B1 × B4, FC2, and B1 × B2 demonstrated positive traits across all traits. Double hybrids (B1 × B4) × (FC1) and (B1 × B2) × (FC1) performed comparably to the control (FC2 × FC1) and showed promising performance. Consequently, these new double hybrids can be further evaluated in field trials and can be considered as viable alternatives to the ruling double hybrid FC2 × FC1.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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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https://www.sdiarticle5.com/review-history/120611