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Estimation of Heterosis in newly evolved Hybrids of Silkworm (*Bombyx mori* L.) at Laboratory condition**(12 Arial)** (Botanical names iItalics)

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| **ABSTRACT(10 Times New Roman): In order to meet the large requirements of the silk industry, different silkworm hybrids, which can sustain in high temperature and humidity, needs to be developed so that they get well adapted under local climatic conditions for stable cocoon production. Thus, there is a need to make new hybrids and to evaluate them for high productivity suitable for this region. In the present study, an attempt was made to evaluate and identify the promising silkworm hybrids raised by involving five multivoltine breeds (BL24, BL67,C. Nichi, Hosa Mysore and MY1) and one bivoltine breed (CSR5) at VNMKV, Parbhani and Dr. PDKV, Akola during 2020-21 and 2021-22 respectively. Observations made for four economically important traits namely; larval weight, single cocoon weight, Cocoon filament length and Cocoon yield/10,000 larvae brushed. The data was subjected to the estimation of heterosis in relation to mid parent value and heterobeltiosis in relation to better parent value revealed that the hybrids namely BL 67 × CSR 5, BL67 × Hosa Mysore and CSR 5 × Hosa Mysore and their respective reciprocals *viz.,* CSR 5 × BL 67, Hosa Mysore × BL 67 and Hosa Mysore × CSR 5 were exhibited significant heterosis and heterobeltiosis for all the four traits studied.****Keywords:** *Bombyx mori*, silkworm, Silkworm breeding, Heterosis, Heterobeltiosis. (At least 5-7) |

**INTRODUCTION(10 Times New Roman)**

Enrichment of silkworm breeds/hybrids have always been one of the important factors contributing to increase the productivity in sericulture sector. Continuous development, evaluation, renewal and change of existing breeds/hybrids with new superior varieties and their commercialization is the prime factor to increase silk quality and quantity with increase in production of cocoon as well as silkworm eggs.

A significant impact of silkworm hybrids through the exploitation of hybrid vigour where introduce through several scientists across the India to increase quantitative and qualitative silk production on a commercial scale and succeeded in the development new silkworm hybrids (Bhat *et al.,* 2017; Murali and Tayal 2018).

The utilization of heterosis is one of the major breakthroughs which contributed to the success of modern silkworm breeding. It refers to the superiority of F1 hybrids in one or more characters over its parents and calculated in percentage. The knowledge of heterosis can give an idea about genetic control of a particular character and may help in elimination of poor crosses in early generation of testing. The exploitation of heterosis through hybridization proved revolutionary in silkworm for economic traits and triggered changes in quantitative and qualitative silk output to maximize the cocoon yield, decrease in larval mortality, increase in filament length. As per available literature, manifestation of heterosis in silkworm has been demonstrated by many breeders (Narayanaswamy *et al.*, 2009; Joshi and Sisodiya 2013). In the present study, an attempt was made to evaluate 30 F1 hybrids obtained by the crossing of 6 parental races of silkworm and identify the most promising hybrids by studding four economic characters of the silkworm

**MATERIAL AND METHODS(10 Times New Roman)**

The experiment was conducted at two different locations, in laboratory condition that was at Sericulture research scheme, Vasantrao Naik Marathwada Krishi Vidyapeeth*,* Parbhani (Maharashtra) during September 2020 to February 2021 (due to pandemic) and Department of Agril. Entomology, PGI, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS), during August 2021 to January 2022.

Disease free layings (DFLs) of six silkworm pure races (five multivoltine races *viz.,* BL-24, BL 67, C. Nichi, Hosa Mysore and MY-1 and one bivoltine race CSR-5) were procured from Central Sericulture Germplasm Resources Centre, Hosure, Tamil Nadu. After emergence the male and female moth by proper identification allowed to mate with different desired combinations of 6 × 6 full diallel system. From this crossing method 30 F1 hybrids and 6 selfed parents were obtained. Again reared these DFLs of F1 hybrids as well as selfed parents separately up to the moth emergence from the cocoons in Randomised Block Design (RBD) with 3 replications. Observations were recorded on these F1 hybrids as well as selfed parents.

During this experiment the cold acid treatment was followed to break the dormancy of eggs laid by bivoltine females with innovative approach. After mating and decoupling, the bivoltine female moths were put separately in to disposable tea cups (made up of plastic coated paper). Each cup was marked with crossing details of that particular female. The egg laying starts soon after the decoupling in a particular disposable tea cups. In next 12 hours the egg laying supposed to be completed, there after the female moths were removed from the cup and disposed off safely. These disposable tea cups containing eggs kept aside for another 8 to 10 hours, after that 20 % concentrated hydrochloric acid was poured into these cups. Ensured that all the eggs in the cup were dipped in the acid. After 1 hour the acid was rinsed out by using plastic sieve followed by washing of eggs thoroughly in tap water to remove the traces of acid remained on egg surface. Then these DFLs were dried and kept for incubation (Fig. 1).

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1. After decoupling female moth kept in disposable cups for eggs laying; 2. Eggs laying of female moth; 3. Deeping of eggs in 20% HCl for 1 hour, at 28°C temperature; 4. Washing of HCl treated eggs under tap water for 5 minutes; 5. Allow eggs to dry by spreading on plane paper for 12 hours.

**Fig. 1.** Cold acid treatment of eggs, laid by bivoltine silkworm females.

*A. Observations noted*

The observations were recorded on 4 economic characters of silkworm, these are as follows.

**1. Larval weight (g):** The maximum silkworm larval weight was recorded by taking the weight of randomly selected 10 matured larvae before the onset of spinning. It was expressed in grams.

**2. Single cocoon weight (g):** The silkworm cocoon weight was recorded on 6th day of spinning, when cocoon weight assumes to be maximum. The average of 10 randomly selected cocoons was taken as single cocoon weight.

**3. Cocoon filament length (m):** Length of silkworm cocoon filament was worked out with the help of epprovate. It is an average of 10 cocoons. The randomly selected cocoons were boiled in water and individual filament length was measured on epprovate.

**4. Cocoon yield/10,000 larvae brushed (kg):** Randomly selected 100 cocoons were weighed and the cocoon yield per 10,000 larvae brushed was computed. It was expressed in kilogram.

*B. Statistical analyses*

**Heterosis.** The data of the above 4 characters were analysed. Hybrid vigor for better parent heterosis and mid parents heterosis (BPH and MPH) were calculated for the selected hybrids following the popular methods (Bhargava *et al*.,1993; Ashoka and Govindan 1990). The per cent MPH and BPH with respect to particular character were calculated in F1 by using the formula.

**RESULTS AND DISCUSSION**

As per the data given in Table 1 to 4revealed the magnitude of heterosis over mid parent *i.e*. heterosis (H1) and heterosis over better parent *i.e*. heterobeltiosis (H2) for four characters was calculated for both the consecutive year 2020-21 and 2021-22 separately as well as pooled data basis. In sericulture, the positive heterosis is desirable for all the four economic characters studied.

**1. Larval weight (g):** The heterosis for larval weight was ranged between -2.74 to 45.45 over mid parent and -21.97 to 38.08 over better parent in the year 2020-21. The reciprocal cross Hosa Mysore × BL 67 (45.45 and 38.08) followed by CSR 5 × BL 67 (44.12 and 21.46), BL 67 × Hosa Mysore (43.00 and 35.76), CSR 5 × Hosa Mysore (39.72 and 12.91), BL 67 × CSR 5 (38.59 and 16.80) recorded highest and significant positive heterosis over mid parent and better parent respectively.

In 2021-22, heterosis was ranged from -1.96 to 36.93 and -18.63 to 28.13 over mid parent and better parent respectively. The reciprocal cross CSR 5 × BL 67 (36.93 and 19.15) followed by Hosa Mysore × BL 67 (35.44 and 28.13), BL 67 × Hosa Mysore (34.89 and 27.61), CSR 5 × Hosa Mysore (34.72 and 11.85) and BL 67 × CSR 5 (32.26 and 15.09) recorded highest and significant positive heterosis and heterobeltiosis respectively.

In pooled analysis, heterosis was ranged between -1.78 to 40.49 over mid parent and -20.31 to 32.95 over better parent. The reciprocal cross CSR 5 × BL 67 (40.49 and 20.31) followed by Hosa Mysore × BL 67 (40.29 and 32.95), BL 67 × Hosa Mysore (38.83 and 31.57), CSR 5 × Hosa Mysore (37.21 and 12.38) and BL 67 × CSR 5 (35.39 and 15.94) recorded highest and significant positive heterosis over mid parent and better parent respectively.

**2. Single cocoon weight (g):** In case of single cocoon weight, the range of mid parent heterosis (-2.55 to 42.58, -2.06 to 40.42 and -0.13 to 41.51) and better parent heterosis (-21.70 to 34.45, -5.16 to 25.72 and -11.48 to 29.66) recorded during 2020-21, 2021-22 and pooled analysis respectively.

During 2020-21, out of thirty hybrids, eleven crosses and thirteen reciprocals recorded significant and positive heterosis over mid parent whereas seven crosses and seven reciprocals recorded significant positive heterosis over better parent. The reciprocal cross CSR 5 × BL 67 (42.48 and 21.04) followed by Hosa Mysore × BL 67 (41.21 and 34.45), BL 67 × Hosa Mysore (40.20 and 33.49), BL 67 × CSR 5 (37.86 and 17.03), CSR 5 × Hosa Mysore (37.36 and 12.02) and Hosa Mysore × CSR 5 (28.15 and 4.51) exhibited maximum and significant positive heterosis over mid parent and better parent respectively.

In 2021-22, out of thirty hybrids twelve crosses and twelve reciprocals recorded significant and positive mid parent heterosis whereas six crosses and five reciprocals exhibited significant positive heterosis over better parent. The reciprocal cross CSR 5 × BL 67 (40.42 and 22.72) followed by Hosa Mysore × CSR 5 (36.13 and 13.10), BL 67 × CSR 5 (35.10 and 18.08), BL 67 × Hosa Mysore (33.41 and 25.72), Hosa Mysore × BL 67 (31.29 and 23.73), and, CSR 5 × Hosa Mysore (30.14 and 8.13) recorded maximum and significant positive heterosis over mid parent and better parent respectively.

In pooled data, out of thirty hybrids, fourteen crosses and fourteen reciprocals exhibited significant and positive mid parent heterosis whereas five crosses and eight reciprocals exhibited significantly positive better parent heterosis. The cross CSR 5 × BL 67 (41.51 and 21.96) followed by BL 67 × Hosa Mysore (36.73 and 29.66), BL 67 × CSR 5 (36.49 and 17.64), Hosa Mysore × BL 67 (35.76 and 28.74), CSR 5 × Hosa Mysore (33.60 and 10.15) and Hosa Mysore x CSR 5 (32.19 and 8.99) exhibited maximum and significant positive mid parent and better parent heterosis respectively.

**3. Cocoon filament length (m):** The heterosis for cocoon filament length was ranged from -7.06 to 61.82, -8.14 to 41.89 and -5.18 to 50.10 over mid parent whereas – 27.42 to 46.44, -20.80 to 29.90 and -23.88 to 37.72 over better parent in 2020-21, 2021-22 and pooled data respectively.

Out of thirty hybrids, 17 hybrids in 2020-21 and 20 hybrids each in 2021-22 and pooled mean data recorded significantly positive heterosis over mid parent. Whereas 6, 7 and 6 hybrids recorded significantly positive heterobeltiosis during 2020-21, 2021-22 and pooled data respectively.

The hybrids BL 67 × CSR 5 (61.82, 28.60 and 44.35), CSR 5 × BL 67 (59.26, 41.89 and 50.10), Hosa Mysore × CSR 5 (48.47, 20.35 and 33.76), Hosa Mysore x BL 67 (48.20,33.20 and 40.34), CSR 5 × Hosa Mysore (41.14,30.46 and 35.51) and BL 67 × Hosa Mysore (37.03,23.87 and 30.17) exhibited significant and maximum positive heterosis over mid parent in both consecutive research trials and pooled analysis respectively. However, the hybrids namely Hosa Mysore × BL 67 (46.44, 29.90 and 37.72), BL 67 × CSR 5 (46.41, 15.72 and 30.25), BL 67 × Hosa Mysore (35.41, 20.80 and 27.74), Hosa Mysore × CSR 5 (32.90, 5.90 and 18.68) and CSR 5 × Hosa Mysore (26.34, 14.80 and 20.24) recorded significant and maximum positive heterobeltiosis in both consecutive research trials and pooled analysis respectively. These are accordance with the results of Joshi and Sisodiya (2013) and Narayanaswamy *et al.* (2009).

**4. Cocoon yield/10,000 larvae brushed (kg):** The heterosis over mid parent for silkworm cocoon yield per 10,000 larvae brushed ranged from -1.35 to 32.11, -3.99 to 26.76 and -1.42 to 28.58 whereas heterosis over better parent ranged between -11.63 to 21.54, -12.59 to 18.08 and -9.83 to 19.26, during 2020-21, 2021-22 and pooled data respectively. Out of thirty hybrids 15, 16 and 23 hybrids for mid parent heterosis whereas 7, 6 and 6 hybrids for better parent heterosis were recorded significantly positive heterosis in 2020-21, 2021-22 and pooled data respectively.

In case of first research trial (2020-21), the significantly maximum and positive mid parent heterosis as well as heterobeltiosis exhibited respectively in the cross BL 67 × CSR 5 (32.11 and 21.54) followed by CSR 5 × BL 67 (29.73 and 19.35), CSR 5 × Hosa Mysore (27.73 and 13.62), Hosa Mysore × BL 67 (21.94 and 17.52) and BL 67 × Hosa Mysore (19.07 and 14.75).

During 2021-22, The cross CSR 5 × Hosa Mysore (26.76 and 11.86), Hosa Mysore x CSR 5 (26.76 and 11.86), Hosa Mysore × BL 67 (25.66 and 18.08), BL 67 × Hosa Mysore (25.38 and 17.82), BL67 × CSR 5 (25.01 and 16.89) and CSR 5 × BL67 (24.43 and 16.34) showed maximum and significant positive heterosis over mid parent and better parent respectively.

In pooled mean data, the cross BL 67 × CSR 5 (28.58 and 19.26) followed by CSR 5 × Hosa Mysore (27.24 and 12.76), CSR 5 × BL 67 (27.09 and 17.88), Hosa Mysore × BL 67 (23.77 and 17.80), BL 67 × Hosa Mysore (22.19 and 16.30) and Hosa Mysore × CSR 5 (21.41 and 7.60) recorded significantly highest and positive mid parent heterosis and heterobeltiosis respectively.

Rao *et al*. (2002) studied heterosis on rearing performance of 25 F1 hybrids and reported that the hybrid BL 67 × CSR5 expressed the highly significant positive heterosis and heterobeltiosis for cocoon yield/10000 larvae by weight, cocoon weight, shell weight and shell ratio. Raghavendra Rao *et al.* (2003) noticed that among twelve F1 hybrids evaluated, four hybrids *viz.,* BL67×CSR4, BL67×CSR5, BL67×NB4D2 and PM×NB4D2 were adjudicated as best heterotic hybrids and recommended for commercial exploitation. Joshi & Sisodiya (2013) noted the significant improvement in fecundity, weight of ten mature larvae and shell percentage ratio in hybrids of Hosa Mysore than the parents. Bhonde *et al.* (2017) studied the seasonal effects on multivoltine × bivoltine and bivoltine × bivoltine silkworm hybrids during rainy, winter and summer seasons in Nagpur region and reported that the multivoltine × bivoltine hybrid gives better production in all the three seasons as compared to bivoltine × bivoltine hybrids. The studies show that the race C. Nichi was recorded superior for denier (1.68) and larval duration (22.96) whereas Hosa Mysore race (16.10%) was found significantly superior over rest of the races for shell ratio. Murali *et al.* (2018) evaluated 5 parental CSR breeds of *Bombyxi mori* L. from the germplasm bank of RSRS, Miran Sahib, regrading phenotypic and biological/economic parameters. The perusal of the data reveals that the highest fecundity was recorded in CSR-5 with 34.94 g weight of ten matured larvae. Sharma and Bukhari (2020) also reported that the existing tropical and sub-tropical situation provides scope for exploiting multivoltine × bivoltine hybrid at commercial venture as they are hardy and have tremendous ability to survive and reproduce under varied or fluctuating environmental climatic conditions.

**Table 1: Estimation of heterosis (H1) and heterobeltiosis (H2) for larval weight (g) of *Bombyx mori* L. (9 Times New Roman)**

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| **Sr. No.** | **Characters** | **Heterosis (H₁)** | **Heterobeltiosis (H₂)** |
| **Crosses** | **2020-21** | **2021-22** | **Pooled** | **2020-21** | **2021-22** | **Pooled** |
| 1. | BL 24 × BL 67 | 4.35 \* | 4.56 \*\* | 4.46 \*\* | -1.34 | -0.92 | -1.13 |
| 2. | BL 24 × C. Nichi | 5.28 \*\* | 4.40 \*\* | 4.84 \*\* | 4.76 \* | 2.1 | 3.39 \*\* |
| 3. | BL 24 × CSR 5 | 0.83 | 4.47 \*\* | 2.66 \*\* | -18.79 \*\* | -13.15 \*\* | -16.00 \*\* |
| 4. | BL 24 × H.M. | 3.81 | 5.17 \*\* | 4.50 \*\* | 3.36 | 4.99 \*\* | 4.37 \*\* |
| 5. | BL 24 × MY1 | 3.88 | 5.31 \*\* | 4.62 \*\* | 2.39 | 4.53 \*\* | 4.28 \*\* |
| 6. | BL 67 × C. Nichi | 15.15 \*\* | 12.55 \*\* | 13.82 \*\* | 8.36 \*\* | 4.42 \*\* | 6.33 \*\* |
| 7. | BL 67 × CSR 5 | 38.59 \*\* | 32.26 \*\* | 35.39 \*\* | 16.80 \*\* | 15.09 \*\* | 15.94 \*\* |
| 8. | BL 67 × H.M | 43.00 \*\* | 34.89 \*\* | 38.83 \*\* | 35.76 \*\* | 27.61 \*\* | 31.57 \*\* |
| 9. | BL 67 × MY1 | 7.82 \*\* | 7.60 \*\* | 7.71 \*\* | 3.36 | 1.24 | 2.27 \*\* |
| 10. | C. Nichi × CSR 5 | -2.74 | -0.37 | -1.55 \* | -21.97 \*\* | -18.63 \*\* | -20.31 \*\* |
| 11. | C. Nichi × H.M | 12.37 \*\* | 9.11 \*\* | 10.70 \*\* | 11.33 \*\* | 6.88 \*\* | 9.03 \*\* |
| 12. | C. Nichi × MY1 | 6.88 \*\* | 5.56 \*\* | 6.23 \*\* | 4.83 \* | 4.00 \*\* | 4.43 \*\* |
| 13. | CSR 5 × H.M | 39.72 \*\* | 34.72 \*\* | 37.21 \*\* | 12.91 \*\* | 11.85 \*\* | 12.38 \*\* |
| 14. | CSR 5 × MY1 | 23.71 \*\* | 19.98 \*\* | 21.84 \*\* | 0.75 | -0.85 | -0.05 |
| 15. | H.M × MY1 | 3.27 | 4.91 \*\* | 4.09 \*\* | 2.23 | 4.31 \*\* | 3.88 \*\* |
| **Reciprocals** |  |  |  |  |  |  |
| 16. | BL 67 × BL 24 | 10.92 \*\* | 8.72 \*\* | 9.78 \*\* | 4.87 \* | 3.02 \*\* | 3.91 \*\* |
| 17. | C. Nichi × BL 24 | 10.97 \*\* | 5.92 \*\* | 8.41 \*\* | 10.42 \*\* | 3.59 \*\* | 6.90 \*\* |
| 18. | CSR 5 × BL 24 | 24.40 \*\* | 21.13 \*\* | 22.76 \*\* | 0.19 | 0.7 | 0.45 |
| 19. | HM × BL 24 | -1.61 | -1.96 | -1.78 \* | -2.04 | -2.12 | -1.91 \* |
| 20. | MY1 × BL 24 | 5.80 \*\* | 3.68 \*\* | 4.72 \*\* | 4.28 | 2.91 \* | 4.38 \*\* |
| 21. | C. Nichi × BL 67 | 10.14 \*\* | 6.50 \*\* | 8.30 \*\* | 3.65 | -1.19 | 1.17 |
| 22. | CSR 5 × BL 67 | 44.12 \*\* | 36.93 \*\* | 40.49 \*\* | 21.46 \*\* | 19.15 \*\* | 20.31 \*\* |
| 23. | HM × BL 67 | 45.45 \*\* | 35.44 \*\* | 40.29 \*\* | 38.08 \*\* | 28.13 \*\* | 32.95 \*\* |
| 24. | MY1 × BL 67 | 16.98 \*\* | 12.81 \*\* | 14.85 \*\* | 12.14 \*\* | 6.14 \*\* | 9.05 \*\* |
| 25. | CSR 5 × C. Nichi | 19.07 \*\* | 17.87 \*\* | 18.46 \*\* | -4.47 \*\* | -3.73 \*\* | -4.11 \*\* |
| 26. | HM × C. Nichi | 7.48 \*\* | 6.29 \*\* | 6.86 \*\* | 6.49 \*\* | 4.12 \*\* | 5.25 \*\* |
| 27. | MY1 × C. Nichi | 5.25 \*\* | 4.35 \*\* | 4.79 \*\* | 3.23 | 2.80 \* | 3.01 \*\* |
| 28. | HM × CSR 5 | 29.43 \*\* | 26.14 \*\* | 27.76 \*\* | 4.58 \*\* | 4.72 \*\* | 4.65 \*\* |
| 29. | MY1 × CSR 5 | -0.14 | 3.03 \*\* | 1.44 \* | -18.68 \*\* | -14.85 \*\* | -16.79 \*\* |
| 30. | MY1 × HM | 13.65 \*\* | 11.12 \*\* | 12.35 \*\* | 12.50 \*\* | 10.48 \*\* | 12.13 \*\* |
| Range of Heterosis (%) | -2.74 to 45.45 | -1.96 to 36.93 | -1.78 to 40.49 | -21.97 to 38.08 | -18.63 to 28.13 | -20.31 to 32.95 |
| SE (m) + | 0.391 | 0.232 | 0.167 | 0.452 | 0.268 | 0.193 |
| CD (5%) | 0.84 | 0.50 | 0.36 | 0.97 | 0.57 | 0.41 |
| CD (1%) | 1.04 | 0.61 | 0.44 | 1.20 | 0.71 | 0.51 |
| \* Significant at 5 % level, \*\*Significant at 1 % level, HM: Hosa Mysore |

**CONCLUSIONS**

By considering the heterosis over mid as well as better parent, three crosses namely BL 67 × CSR 5, BL67 × Hosa Mysore and CSR 5 × Hosa Mysore and their respective reciprocals *viz.,* CSR 5 × BL 67, Hosa Mysore × BL 67 and Hosa Mysore × CSR 5 were identified as the promising.

**FUTURE SCOPE**

The promising hybrids identified in this study can be exploited commercially to increase silk quality and quantity with increase in production of cocoon.

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**Conflict of interest.** None. **(9 Times New Roman)**

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