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# Estimation of Heterosis in Newly Evolved CSR Bivoltine Hybrids of Silkworm (*Bombyx mori* L.) at Room and High Temperature

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**Abstract**

Silkworm breeds/hybrids which are reared over a series of environment exhibiting less variation are considered stable. Identification of high temperature tolerant bivoltine breeds/hybrids by screening for thermo tolerance in silkworm is an essential prerequisite for the development of thermo-tolerant bivoltine breeds/hybrids. One of the main aims of the breeders is to recommend breeds/hybrids that are stable under different environmental conditions and minimize the risk of falling below a certain yield level to farmers. In the present study, an attempt was made to evaluate and identify thermo-tolerant, adaptable bi×bivoltine silkworm hybrids suitable for rearing throughout the year, raised by involving seven productive breeds (CSR2, CSR3, CSR4, CSR5, CSR6, KA and $NB_4D_2$) and two thermo-tolerant breeds (CSR18 and CSR19) at two different temperature regime (25±1 °C and 36±1 °C ). The data was subjected to the estimation of heterosis in relation to mid parent value and heterobeltiosis in relation to better parent value. Observations made for four economically important traits namely; pupation percentage, single cocoon weight, single shell weight and shell ratio percentage revealed that at 25±1 °C (optimum environmental conditions) productive hybrid CSR3×CSR6 (heterosis over mid parent value (66.99) and heterobeltiosis over better parent value (50.62) results in better qualitative and quantitative traits as compared to other hybrids studied. Robust hybrid, CSR18×CSR19 showed better tolerance to high temperature i.e. at 36±1 °C and recorded heterosis for mid parent value of 55.03 and heterobeltiosis for better parent value of 32.40 when compared to productive hybrids.

***Keywords:*** Heterosis, heterobeltiosis, hybrid vigour, thermotolerant, adaptable

## 1. Introduction

The Indian sericulture industry depends mainly on multi/polyvoltine races/hybrids. India is now on the threshold of vitalizing the silk industry with greater emphasis on quality rather than quantity. With the gradual shifting from quality to quantity, the bivoltine races are now favoured prima bonnas. The change in the attitude is mainly attributed to the fact that the quality of silk produced by the polyvoltine races, traditionally reared in India, is rather poor when measured by the international silk standards. The only major factor in favour of the polyvoltines seems to be that they are hardy and have tremendous ability to survive and reproduce under varied or fluctuating environmental conditions (Chandrakanth et al., 2015). This vulnerability is more pronounced in bivoltine breeds as compared to poly/multivoltine breeds. Thus, among many factors responsible for poor performance of the bivoltine breeds under tropical conditions, the major one is lack of thermo tolerance (Kumari et al., 2011). Many quantitative characters decline sharply at higher temperature. Therefore, high temperature is one of the main factors for bivoltine crop failure in the tropics. Thus, farmers restrict themselves to rear silkworm breeds/hybrids suitable for high temperature conditions prevailing in the field and this prompts the commercial exploitation of multi×bivoltine hybrids in India, as they are hardy and able to survive and reproduce under fluctuating climatic conditions (Kumari et al., 2011). The quantity of silk produced by multi×bivoltine hybrids meet the domestic needs but the quality of silk is low when compared to bivoltine silk of international grade. High quality, internationally recognized silk production can be achieved only by rearing bivoltine breeds/hybrids. To overcome this drawback, compatible bivoltine breeds/hybrids for rearing under tropical conditions were developed (Lakshmi et al., 2011) and selected for rearing in field conditions. However, the attempts to spread bivoltine silkworm breeds/hybrids throughout the sericulture belt of India resulted in extensive crop loss, especially in the hot and humid climatic conditions of tropics (Chavadi et al., 2006). These productive hybrids were vulnerable to varied environmental conditions, as they originated from temperate regions. In order to introduce bivoltine races/hybrids in tropical areas, it is necessary to have stability in cocoon crop under high temperature environments. Considering the poor performance of bivoltine

 

races/hybrids during summer season, emphasis was given to evolve bivoltine silkworm breeds/hybrids suitable for tropical conditions for achieving the primary objective of establishing bivoltine sericulture with quality raw silk among sericulturists. To overcome this, the development of productive bivoltine breeds/hybrids under varied environmental conditions is imperative for introducing bivoltine crop for commercial exploitation.

## 2. Materials and Methods

The rearing of silkworms were carried out in a Completely Randomized Block Design (CRD) during August-September, 2015, October-November, 2015 and December, 2015-January, 2016 as per the standard rearing techniques of Krishanaswamy (1988) till 2nd day of 5th instar. The experimental research material for the proposed study comprised of four newly evolved bivoltine silkworm hybrids viz., CSR2×CSR4, CSR2×CSR5, CSR3×CSR6 and CSR18×CSR19 along with traditional bivoltine hybrid, KA×$NB_4D_2$ and their parental breeds. Seven parental breeds, CSR2, CSR3, CSR4, CSR5, CSR6, KA and $NB_4D_2$ were developed as productive breeds which are high yielding and qualitatively superior, where as CSR18 and CSR19 were evolved as robust breeds tolerant to high temperature. The salient features of the parental breeds are presented in Table 1. The breeds were crossed and $F_1$ seed of hybrid combinations was prepared to develop, evaluate and identify new bi×bivoltine hybrids for achieving the primary objective of establishing bivoltine hybrids tolerant to high temperature. Three trails with three replicates each were conducted for this study at both room (25±1 °C) and high (36±1 °C) temperature conditions. With regard to thermal exposure, the 3rd day of 5th instar larvae were reared in a SERICATRON (environment chamber with precise and automatic control facilities for uniform maintenance of temperature and humidity) with an exposure duration of six hours (10:00–16:00) $day^{-1}$ till spinning. After thermal treatment, the larvae were shifted to 25±1 °C daily till spinning. The observations were recorded replication-wise for different parameters viz., pupation percentage, single cocoon weight, single shell weight and shell ratio percentage and subjected to the estimation of heterosis in relation to mid parent value and heterobeltiosis in relation to better parent value (Shull, 1948).

Table 1: Salient features of parental breeds

| Breeds | Fecundity | Newly laid egg colour | Newly hatched larvae colour | Larval pattern | Cocoon shape and colour |
|---|---|---|---|---|---|
| CSR2 | 450-500 | Deep yellow | Deep brown | Plain | Oval, white |
| CSR3 | 450-475 | Deep yellow | Black | Sex-limited | Oval, white |
| CSR4 | 475-500 | Light yellow | Black | Plain | Dumb-bell, white |
| CSR5 | 475-525 | Deep yellow | Black | Plain | Dumb-bell, white |
| CSR6 | 475-525 | Deep yellow | Black | Marked | Dumb-bell, white |
| CSR18 | 450-475 | Deep yellow | Black | Sex-limited | Oval, white |
| CSR19 | 450-475 | Deep yellow | Black | Sex-limited | Dumb-bell, white |
| KA | 470-650 | Light yellow | Black | Plain | Oval, white |
| $NB_4D_2$ | 475-550 | Deep yellow | Deep brown | Plain | Dumb-bell, white |

### *2.1. Heterosis*

It was measured as the $F_1$ deviation from mid parental value. Heterosis was obtained by using following formula:

Heterosis (%)=(H–MPV/MPV)×100

Where, H=Mean performance of $F_1$ hybrid

MPV=Mid parental value (P1+P2)/2

### *2.2. Heterobeltiosis*

It was measured as the $F_1$ deviation from better parental value. Heterobeltiosis was obtained by using following formula:

Heterobeltiosis (%)=(H–BPV/BPV)×100

Where, H=Mean performance of $F_1$ hybrid

BPV=Better parental value

## 3. Results and Discussion

### *3.1. Mean performance of hybrids*

Since the three trails of hybrid rearing were conducted under controlled environmental conditions, no variations on the performance between trails were noticed. The deleterious effect of high temperature was so pronounced on the productive breeds as indicated by 100% mortality compared to the robust breeds as evidenced by their better performance (Table 2). However, the deleterious effect was not pronounced on productive and robust hybrids. The mean value of hybrids for four characters namely pupation percentage, single cocoon weight, single shell weight and shell ratio percentage are presented in Table 3. With regard to pupation percentage at 25±1 °C, it ranged from 92.26–97.82% with the highest of 97.82% recorded for CSR3×CSR6 and the lowest for hybrid KA×$NB_4D_2$ (92.26%). Highest pupation rate at 36±1 °C was recorded by CSR18×CSR19 (68.90%) and lowest for control hybrid KA×$NB_4D_2$ (37.00%). At 25±1 °C, the highest single cocoon weight was recorded for KA×$NB_4D_2$ (2.27 g) followed by CSR3×CSR6 (2.18 g) and CSR2×CSR4 (2.07 g). At 36±1 °C, the highest single cocoon weight was again expressed by hybrid KA×$NB_4D_2$ (1.63 g) and lowest in CSR3×CSR6 (1.32 g). At 25±1

Table 2: Rearing performances of breeds at two different temperature treatments

| Breeds/Hybrids | Pupation percentage | | Single cocoon weight (g) | | Single shell weight (cg) | | Shell ratio percentage | |
|---|---|---|---|---|---|---|---|---|
| | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C |
| Robust breeds | | | | | | | | |
| CSR18 | 89.53 | 65.00 | 1.78 | 1.45 | 41.40 | 29.90 | 23.30 | 20.60 |
| CSR19 | 87.67 | 62.50 | 1.66 | 1.24 | 36.90 | 24.30 | 22.20 | 19.60 |
| Productive breeds | | | | | | | | |
| CSR2 | 92.54 | - | 1.85 | - | 44.70 | - | 24.20 | - |
| CSR4 | 91.52 | - | 1.71 | - | 37.20 | - | 21.80 | - |
| CSR5 | 94.17 | - | 1.81 | - | 41.90 | - | 23.10 | - |
| CSR3 | 86.73 | - | 1.83 | - | 44.50 | - | 24.30 | - |
| CSR6 | 87.63 | - | 1.73 | - | 38.70 | - | 22.40 | - |
| KA | 84.38 | - | 1.92 | - | 39.30 | - | 20.50 | - |
| $NB_4D_2$ | 83.55 | - | 1.68 | - | 35.90 | - | 21.36 | - |

Table 3: Rearing performances of hybrids at two different temperature treatments (Mean of I, II & III Trials)

| Characters → Hybrids ↓ | Pupation percentage | | Single cocoon weight (g) | | Single shell weight (cg) | | Shell ratio percentage | |
|---|---|---|---|---|---|---|---|---|
| | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C |
| CSR18×CSR19 | 95.89 | 68.90 | 1.82 | 1.45 | 44.70 | 33.30 | 24.56 | 22.96 |
| CSR2×CSR4 | 96.96 | 38.64 | 2.07 | 1.46 | 50.20 | 31.80 | 24.30 | 21.70 |
| CSR2×CSR5 | 95.50 | 42.73 | 2.02 | 1.36 | 49.20 | 29.90 | 24.40 | 22.00 |
| CSR3×CSR6 | 97.82 | 37.81 | 2.18 | 1.32 | 53.00 | 27.80 | 24.50 | 21.10 |
| KA×$NB_4D_2$ | 92.26 | 37.00 | 2.27 | 1.63 | 47.70 | 32.30 | 21.00 | 19.80 |
| SEm± | 0.01 | 0.01 | 0.00 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 |
| LSD *(p=0.05)* | 0.04 | 0.03 | 0.00 | 0.02 | 0.07 | 0.02 | 0.02 | 0.02 |

°C, the single shell weight was highest for CSR3×CSR6 (53.00 cg) and the lowest of 44.70 cg recorded for CSR18×CSR19. At 36±1 °C, the single shell weight of 33.30 cg was recorded highest for CSR18×CSR19 and the lowest of 27.80 cg recorded for CSR3×CSR6. The highest shell ratio percentage at 25±1 °C was recorded by CSR18×CSR19 (24.56%) and the lowest of 21.00% was recorded for control hybrid, KA×NB4D2. Highest shell ratio percentage at 36±1 °C was recorded by CSR18×CSR19 (22.96%) followed by CSR2×CSR5 (22.00%) and the lowest of 19.80% recorded by hybrid, KA×$NB_4D_2$.

*3.2. Nature and extent of heterosis and heterobeltiosis in CSR silkworm hybrids*

Heterosis and Heterobeltiosis were estimated under two temperature schedules of 25±1 °C and 36±1 °C in newly evolved CSR bivoltine hybrids of *Bombyx mori* L. The estimates of heterosis over mid and better parents for different temperature treatments have been presented in Table 4 and 5. The productive parental breeds of hybrids did not survive at higher temperature i.e. 36±1 °C, the heterosis and heterobeltiosis percentage of hybrids could be estimated only at 25±1 °C except for robust parental breeds and its hybrid. The degree and direction of heterosis varied for different hybrids under different environmental conditions (Rashid et al., 2011). Though, the phenomenon of hybrid vigour was exploited in plants especially in maize much earlier to that of silkworm, the extent of exploitation in silkworm is unparalleled to any of the commercial crops. Ultimate objective of silkworm breeding is not only to synthesize new genotypes but also to identify sustainable silkworm hybrids for commercial purpose. In order to improve the yield and quality of silk, it is pertinent to characterize the potential of available genetic resources for transferring the desirable components in hybrids. Selection of parents on the basis of *per se* performance does not always lead to fruitful results. More diverse the parents more are the chances of heterosis (Talebi et al., 2009). The earnest efforts of silkworm breeders have resulted in the evolution of large no. of silkworm strains expressing well-defined qualitative and quantitative traits (Sajgotra et al., 2016).

Pupation rate is one of the important economic characters to determine the variability of breed/hybrids. It is perhaps an independent character but is greatly dependent on rearing environment, food quality and other abiotic factors and is a positive sign for cocoon reeling performance as well as seed production. The genetic and environment factor gets more

Table 4: Heterosis percentages of CSR hybrids at two different temperature treatments (Mean of I, II & III Trials)

| Hybrids → Characters ↓ | CSR18×CSR19 | | CSR2×CSR4 | | CSR2×CSR5 | | CSR3×CSR6 | | KA×$NB_4D_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C |
| Pupation percentage | 8.22 | 8.07 | 5.35 | - | 2.30 | - | 12.20 | - | 9.88 | - |
| Single cocoon weight | 5.81 | 9.85 | 16.20 | - | 10.38 | - | 22.47 | - | 26.11 | - |
| Single shell weight | 14.18 | 22.88 | 22.50 | - | 13.62 | - | 27.40 | - | 26.86 | - |
| Shell ratio percentage | 7.96 | 14.23 | 5.60 | - | 4.66 | - | 4.92 | - | 0.23 | - |
| Cumulative heterosis | 36.17 | 55.03 | 49.65 | - | 30.96 | - | 66.99 | - | 63.08 | - |

Table 5: Heterobeltiosis percentages of CSR hybrids at two different temperature treatments (Mean of I, II & III Trials)

| Hybrids → Characters ↓ | CSR18×CSR19 | | CSR2×CSR4 | | CSR2×CSR5 | | CSR3×CSR6 | | KA×$NB_4D_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C | 25±1 °C | 36±1 °C |
| Pupation percentage | 7.10 | 6.00 | 4.77 | - | 1.41 | - | 11.60 | - | 9.33 | - |
| Single cocoon weight | 2.24 | 3.57 | 1.89 | - | 9.18 | - | 19.10 | - | 18.20 | - |
| Single shell weight | 7.97 | 11.37 | 12.30 | - | 10.06 | - | 19.10 | - | 21.37 | - |
| Shell ratio percentage | 5.41 | 11.46 | 0.41 | - | 0.82 | - | 0.82 | - | -1.86 | - |
| Cumulative heterobeltiosis | 22.72 | 32.40 | 19.37 | - | 21.47 | - | 50.62 | - | 47.04 | - |

reflected in this character. In the present study, estimates of heterosis over mid and better parents for pupation percentage was maximum in hybrid CSR3×CSR6 (12.20, 11.60) followed by KA×$NB_4D_2$ (9.88, 9.33), whereas, minimum value for this trait was recorded in hybrid CSR2×CSR5 (2.30, 1.41) at 25±1 °C, whereas, at 36±1 °C only CSR18×CSR19 (12.20, 6.00) exhibited positive heterosis over both mid and better parents for this trait. The higher pupation percentage in these hybrids can be attributed to the fact that they were more adaptable to different seasons and can also with stand the higher temperature and humidity and thus the variations observed in pupation percentage between hybrids during different seasons can be attributed to the influence of environment on silkworm. Pupation rate is a low heritable character and is prone to large variations in different environmental conditions and management. The observations are in accordance with the findings of Gowda et al. (2013).

Minagawa and Otsuka (1975) have reported inter relationship between multiple characters in silkworm. It therefore, becomes essential to evaluate the breeds and their hybrids to understand the magnitude of heterosis towards improvement in cocoon and silk productivity (Bandopadyay, 1990). Economic characters like cocoon yield, cocoon weight, cocoon shell weight and cocoon shell percentage are inter-related and moreover they influence the productivity. The cocoon weight, shell weight and shell ratio are the important commercial parameters. The cocoon weight has a negative correlation with shell ratio but positive correlation with shell weight, where as shell weight has a positive correlation with shell ratio.

Single cocoon weight is an important commercial parameter for the production of both quality and quantity of cocoon crop. The maximum heterosis value was recorded in hybrid KA×$NB_4D_2$ (26.11) followed by CSR3×CSR6 (22.47), whereas, minimum values (5.81) was recorded in hybrid CSR18×CSR19 respectively at 25±1 °C. However, maximum heterosis for single cocoon weight was recorded in hybrid CSR18×CSR19 (9.85) at 36±1 °C. Heterosis over better parents was highest in CSR3×CSR6 (11.60) and lowest in CSR2×CSR4 (1.89) at 25±1 °C. At 36±1 °C, highest value of heterosis was recorded for CSR18×CSR19 (1.89). Single shell weight is the silk contributing parameter. The maximum heterosis value over mid parent for this parameter was found in hybrid CSR3×CSR6 (27.40) closely followed by KA×$NB_4D_2$ (26.86), whereas, it was minimum in hybrid CSR2×CSR5 (13.62) at 25±1 °C. The heterotic hybrid at 36±1 °C was found to be CSR18×CSR19 (22.88) respectively. Higher heterosis values for cocoon and shell weight may be due to the superiority of these hybrids, thus indicating the phenomenon that heterosis could be either due to additive gene action or due to dominance (Petkov et al., 2007). The results are in accordance with the findings of Sadaphal et al. (2015). Shell ratio percentage is an important parameter of quality depicting actual silk content of a cocoon. The maximum heterosis value was recorded in hybrid CSR18×CSR19 (7.96) closely followed by CSR2×CSR4 (5.60) and CSR3×CSR6 (4.92) at 36±1 °C. The heterotic hybrid found for this parameter at 36±1 °C was CSR18×CSR19 (14.23) respectively. Heterosis value over better parent at 25±1 °C was recorded maximum by CSR18×CSR19 (5.41). Same heterotic value was recorded in two hybrids CSR2×CSR5 and CSR3×CSR6 (0.82) respectively. Negative heterobeltiosis of -1.86 was observed by KA×$NB_4D_2$ for this trait. CSR18×CSR19 (11.46) again recorded maximum value over better parent at 36±1 °C for shell ratio percentage. The high heterosis values obtained for bivoltine hybrids compared can be ascribed to its genetic constitution. The results are in close conformity with the observations of

Subba Rao and Sahai (1989) who reported the additive gene action for this trait. Sadaphal et al. (2015) has also reported the superiority of mulberry varieties particularly triploids responsible for higher cocooning characters. Similar findings were also recorded by Kumari et al. (2011). Better degree of heterosis and heterobeltiosis is recorded in almost all the hybrids taken for study. It is clear from the results that heterotic values are found superior in all the hybrids at 25±1 °C and 36±1 °C. On the other hand, heterobeltiotic values are also found better in all the hybrids than their parents both at 25±1 °C and 36±1 °C respectively. However, it a negative value of -1.86 was noticed in hybrid KA×$NB_4D_2$ at 25±1 °C for shell ratio percentage. This negative value may be due to higher value for that character in the parents involved and therefore, the values have considerably reduced. It is well said that, when the improvement of a particular trait is high in the parental strains, the degree of heterosis declines in the hybrids and vice versa. It is clear from the results that higher the values of the character in parental strains than hybrids lower the heterotic values.

Sajgotra et al. (2016) suggested that the superiority and potential of a breed and hybrids mainly depend on the ranking and considering all the major cocoon yield parameters. Thus, based on these facts it can be concluded that the estimates for heterosis and heterobeltiosis worked out for five hybrids for commercial parameters were pooled. The perusal of data on the basis of cumulative values (Table 4 and 5) of different traits revealed that, among the productive hybrids considered, increased hybrid vigour over mid parent value (66.99) and better parent value (50.62) was well manifested in CSR3×CSR6 at 25±1 °C and stood 1st. Further the present study clearly indicates the robust hybrid, CSR18×CSR19 showed better tolerance to high temperature i.e. at 36±1 °C and recorded mid parent value of 55.03 and better parent value of 32.40 when compared to productive hybrids.

## 4. Conclusion

Hybrid CSR3×CSR6 results in better qualitative and quantitative traits at 25±1 °C for heterosis and heterobeltiosis compared to other hybrids studied. Robust hybrid, CSR18×CSR19 was found to be best that can perform well to obtain higher productivity under varied agro-climatic conditions of the tropics with optimum qualitative and quantitative characteristics and thus can lead to better growth and development of silkworms, which in turn leads to higher crop productivity.

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