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Heterotic Expression in Silk Productivity of Newly Evolved Thermo-tolerant Bivoltine Hybrids of Silkworm *Bombyx mori* L.

P. Sudhakara Rao*, R. K. Datta[1], K. M. Vijaya Kumari, A. K. Palit and S. Z. Haque Rufaie[2]
Central Sericultural Research and Training Institute, Mysore-570008, India.
[1]Director (Retired), No: 202, 8th cross, Srirampura-II stage, Mysore-570023, India.
[2]Division of Sericulture, S. K. University of Agricultural Sciences and Technology, Kashmir-190001, India.

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Studies on heterotic expression in the silk productivity of 66 thermo-tolerant bivoltine hybrids reared under high temperature (36 ± 1°C) and low humidity (60 ± 5%) conditions revealed that manifestation of heterosis was highly significant for majority of the quantitative traits contributing to more cocoon productivity. Observations were made on 8 economically important traits such as fecundity, pupation rate, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, cocoon filament length and raw silk. Analysis of variance results revealed great deal of variability indicating significant ($P < 0.001$) variations. Out of 66 hybrids, 7 hybrids exhibited positive heterosis for seven traits and 4 hybrids expressed significant heterobeltiosis for 6 traits, out of 8 traits evaluated. Based on results, the hybrids $SR_1 \times SR_5$ and $CSR_2 \times SR_5$ are adjudged as most promising ones and can be exploited commercially in tropical climates to increase the cocoon productivity.

Key words: *Bombyx mori* L , Hybrid vigour, heterosis and heterobeltiosis

Introduction

The earnest efforts of silkworm breeders have resulted in the evolution of large number of silkworm strains expressing well defined qualitative and quantitative traits. The introduction of hybrid concept for greater productivity and evaluation of the F_1 hybrid combinations derived from the selected pure lines is undoubtedly the most widely tested method for identifying the superiority of the hybrid. Even though the parental strains are superior and if they do not reflect their desirable economic traits in F_1 population, they donít add much value. The superiority of hybrids over parental strains with regard to commercial traits is due to high magnitude of heterosis for most of the quantitative traits (Hirobe and Ohi, 1954; Harada, 1961; Gamo, 1976; Gamo and Hirabayashi, 1983).

Conventional breeding methods are directed not only to synthesize new breeds but also to identify the most promising hybrid combinations for commercial exploitation based on the heterosis and hybrid vigour expressed in their F_1 population. The silkworm breeds developed for tropical countries like India has to adapt to both seasonal and local conditions to have stability in cocoon crop under high temperature environment. Thus an attempt has been made to analyse heterosis and hybrid vigour in the newly evolved thermo-tolerant bivoltine hybrids of the silkworm under simulated tropical conditions through diallel cross method to select the best hybrids for rearing throughout the year.

Materials and Methods

The newly evolved thermo-tolerant lines SR_1, SR_2 and SR_3 characterized by plain larvae spinning white oval cocoons, dumbbell lines SR_4, SR_5 characterized by marked larvae and SR_6 characterized by plain larvae spinning white peanut shaped cocoons and popular bivoltine strains *viz.*, KA, CSR_2, CSR_{18}, characterized by plain larvae spinning oval cocoons, (CSR_{18} with sex-limited marked and plain larvae). The popular dumbbell breeds, NB_4D_2, CSR_4, characterized by plain larvae and CSR_{19} characterized by sex-limited marked and plain larvae were

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*To whom correspondence should be addressed.

Central Sericultural Research and Training Institute, Mysore-570008, India. Tel: +91-821-2362571; Fax: +91-821-362845; E-mail: parimi_rao @ yahoo.com

utilized in the present study to make all possible combinations of hybrids by employing the diallel cross technique out lined by Griffing (1956; model-2) KA × NB_4D_2 hybrid was kept as control. These 12 breeds were utilized in 12 × 12 diallel crossing system and a total of 66 hybrids were derived to evaluate heterosis and over dominance in order to understand the genetic potentiality of the new lines and for identifying most promising hybrids. Data were collected on eight economic traits such as fecundity, pupation rate, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length and raw silk (%). All the hybrids along with their parents were reared in three mass replicates. After passing third moult 300 larvae per each replication were taken and 100 larvae from each replication were separated for high temperature treatment as out lined by Suresh Kumar *et al.* (2001). After passing fourth moult, on 2nd day 100 larvae each of three replicates were subjected to high temperature (36 ± 1°C) and low humidity (60 ± 5% R.H.) conditions in SERICATRON (A small environmental chamber having precise mechanism to maintain required temperature and humidity automatically - made in Japan) for 6 hrs daily from 10 A.M. to 4 P.M. till the completion of spinning. The remaining larvae were reared at room temperature (25 ± 1°C; Relative humidity 70 ± 5%) as per the procedure outlined by Krishaswami (1978). The data pertaining to eight economic traits *viz.,* fecundity, pupation rate, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length and raw silk percent in treated batches reared under high temperature and low humidity conditions were only considered and subjected to relevant statistical methods.

The data for CRD (Completely Randomized Design) was analyzed by the standard statistical procedure of Diallel analysis as mentioned above. The data pertaining to the expression of Heterosis and over dominance in hybrids with regard to eight economic traits of importance were studied by employing the following formulae (Harada, 1961).

$$\text{Heterosis (\%)} = \frac{\text{Mean of the hybrid } (F_1) - \text{Mid-parent value (MPV)}}{\text{Mid-parent value (MPV)}} \times 100$$

$$\text{Mid Parent Value} = \frac{\text{Mean of parent one + Mean of the parent two}}{2}$$

$$\text{Over dominance (OD) (\%)} = \frac{F_1 - \text{Better parent value (BPV)}}{\text{Better parent value (BPV)}} \times 100$$

Where,

H % = Percentage of heterosis
OD = Percentage of over dominance
F_1 = Mean value of the hybrid
MP = Mean value of the mid parent.
BP = Mean value of the better parent

The standard errors of testing of heterosis and over dominance were calculated as S.E. Heterosis $= \frac{\sqrt{3} \times EMS}{2 \times r}$

S.E. Overdominance $= \frac{\sqrt{2} \times EMS}{r}$

where,

EMS is the Error Mean square of the Completely Randomized Design and ‘r’ is the number of replications.

CD = SE × t value at 5% or 1% level for error degree of freedom.

Results

The mean data on the performance pertaining to eight quantitative traits of economic importance of 12 parental breeds and their selected hybrids derived through diallel cross technique is presented in Tables 1 and 2 respectively. While the data pertaining to heterosis and overdominance are presented in Tables 3 and 4.The results of Analysis of variance computed for 8 economic traits studied for the above crosses indicating the source of variations due to replicates, treatments, parents, hybrids, parents Vs hybrids are presented in Table 5.

The details pertaining to the mean performance of the pure breeds, their hybrids, heterosis and overdominance of the parents and hybrids with respect to each one of the 8 economic traits evaluated are summarized as follows;

Fecundity: Mean values of fecundity of the hybrids revealed great variability to a extent of 508 eggs (SR_1 × CSR_{19}) and a maximum of 592 (SR_1× SR_6). Out of 66 hybrids studied, 39 hybrids have exhibited above over all mean of 549 (Table 2). The heterosis calculated for this trait was observed to exhibit positive values varying between 4.4 to 12.1% (Table 3). Out of 66 hybrid combinations, 17 were found to exhibit significant ($P < 0.05$) better parent heterosis for majority of the traits evaluated (Table 4).

Pupation rate (%): The mean values of hybrids for pupation rate revealed high variability to a great extent with a minimum of 54.3% (KA × CSR_4) and to a maximum of 92.3% (SR_1 × SR_4). Out of 66 hybrids studied, 45 hybrids were found to exhibit higher mean values than the over all mean of 82.0% (Table 2). The heterosis calculated for this trait was observed to record positive values ranging between 3.6% to 40.7% (Table 3). Out of 66 hybrid com-

Table 1. Mean performance of parental breeds utilised in diallel crosses

Parental breeds	Fecundity (no)	Pupation rate (%)	Cocoon yield (Kg)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Raw silk (%)
SR1	530	86.6	12.51	1.68	34.9	21.9	865	14.63
SR2	528	84.0	12.25	1.73	39.0	22.5	953	14.92
SR3	513	91.3	14.44	1.74	38.1	21.7	901	14.81
KA	547	53.0	8.40	1.50	31.2	20.8	797	12.11
CSR18	544	84.0	13.64	1.62	35.8	22.0	894	15.14
CSR2	563	44.6	6.66	1.44	35.4	24.5	723	12.03
SR4	515	82.0	12.81	1.52	34.5	22.5	791	14.80
SR5	526	75.6	12.08	1.58	35.8	22.4	855	15.02
SR6	536	75.6	11.99	1.58	34.3	21.6	785	14.02
CSR4	534	57.6	9.83	1.52	37.0	24.1	888	14.37
NB4D2	511	47.6	7.67	1.60	32.4	20.2	668	11.95
CSR19	514	87.0	12.69	1.59	34.0	20.8	859	14.16
Mean	530	72.4	11.23	1.59	35.0	22.1	832	14.00
SD	18.87	16.54	2.45	0.100	0.020	1.30	79.82	1.22
CV%	3.56	22.84	21.80	6.13	6.53	5.87	9.60	8.72

Table 2. Mean performance of 5 selected hybrids for 8 traits of economic importance

No hybrids	Fecundity (no)	Pupation rate (%)	Cocoon yield (Kg)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Raw silk (%)
1. SR1 × SR4	546	92.3	16.53	1.83	42.1	22.9	1177	18.10
2. SR1 × SR5	565	91.3	16.60	1.75	41.5	23.5	1143	17.62
3. CSR2 × SR5	577	80.0	14.24	1.77	40.2	22.5	1025	16.92
4. SR4 × CSR4	577	86.3	15.33	1.77	39.2	22.0	973	17.48
5. SR4 × CSR19	565	91.6	14.52	1.71	37.2	21.6	901	16.70
Mean	566	88.3	15.44	1.76	40.0	22.7	1043	17.36

Table 3. Heterotic effects(MPV) of 7 selected hybrids for 8 traits of economic importance

No hybrids	Fecundity	Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio	Filament length	Raw silk
1. SR1 × SR4	4.59*	9.27**	28.11**	3.94**	144.02**	3.07	42.15**	21.46**
2. SR2 × CSR4	0.06	4.82*	34.86**	7.81**	152.32**	2.71	24.16**	13.94**
3. SR2 × SR5	0.13	14.41**	37.39**	2.82*	136.72**	3.15	18.93**	20.38**
4. SR3 × SR6	7.50**	9.60 **	20.97**	3.19**	145.68**	4.32	31.38**	23.38**
5. CSR2 × SR4	6.22**	29.66**	40.00**	33.18**	119.69**	-3.29	25.98**	25.18**
6. CSR2 × NB4D2	3.66	38.13**	32.92**	16.60**	128.37**	10.70*	52.89**	31.45**
7. SR4 × NB4D2	11.44**	15.68**	23.33**	-5.76**	124.08**	-0.64	23.46**	19.02**
SE	12.044	1.642	0.27962	0.01	0.005	0.955	13.435	0.2405
CD at 5%	23.792	3.245	0.5524	0.0419	0.011	1.887	26.541	0.4751

*Significant at 5%, **Significant at 1%.

binations, 24 hybrids were found to exhibit significant ($P < 0.05$) better parent heterosis for majority of the traits evaluated (Table 4).

Cocoon yield (kg): The mean values of cocoon yield of the hybrids revealed a wide range of values with a lowest of 7.74 kg (KA x NB_4D_2) and 17.37 kg (SR_3 x SR_5). Out of 66 hybrids studied, 41 hybrids have recorded highest mean values than the over all mean of 14.42 kg (Table 2). The heterosis calculated for this trait was observed to exhibit positive values varying between 9.7% to 58.6%

Table 4. Better parent heterosis (BPV) values of 5 selected hybrids for 8 traits of economic importance

No hybrids	Fecundity	Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio	Filament length	Raw silk
1. SR1 × SR4	3.08	6.13**	27.18**	-8.30**	22.01**	1.74	36.07**	17.24**
2. SR1 × SR5	6.54*	4.96*	27.70**	-12.12**	15.94**	4.78	32.10**	15.87**
3. CSR2 × SR5	2.43	5.73*	17.82**	11.93**	12.30**	-5.94	19.80**	22.37**
4. SR4 × CSR4	8.05**	5.28*	17.95**	-11.20**	5.86**	-8.86	9.49**	11.31**
5. SR4 × CSR19	9.71**	5.36*	11.69**	-14.25**	9.31**	-5.77	4.85**	9.60**
SE	13.9072	1.8969	17.465	0.0245	0.0068	1.1032	15.5136	0.2777
CD at 5%	27.4735	3.7473	5.645	0.0484	0.0314	2.1794	30.6470	0.5486

*Significant at 5%, **Significant at 1%.

(Table 3). Out of 66 hybrid combinations, 53 hybrids were found to exhibit significant ($P < 0.05$) better parent heterosis for this trait. (Table 4).

Cocoon weight (g): The mean values of cocoon weight of the hybrids revealed a wide range with the lowest mean values for hybrids recorded 1.47 g (KA x NB_4D_2) and a maximum of 1.90 g (SR_2 x SR_4). Out of 66 hybrids studied, 40 hybrids were found to exhibit higher mean values than the total mean of 1.73 g for this trait (Table 2). The heterosis calculated for this trait was observed to exhibit positive values varying between 2.6% to 37.4 % (Table 3). Out of 66 hybrid combinations, 4 hybrids were (CSR_2 x SR_5, CSR_2 x SR_4, CSR_2 x CSR_4 CSR_2 x CSR_{19}) found to exhibit significant better parent heterosis ($P < 0.05$) for this trait. On the other hand the values for 4 hybrids were found to exhibit significant over dominance (Table 4).

Cocoon shell weight (g): The mean values of 66 hybrids revealed a wide range of values between 0.30g (KA x NB_4D_2) to a maximum value of 0.43 (SR_2 x SR_4). Out of 66 hybrids studied, 29 hybrids have exhibited above over all mean of 0.38 g (Table 2). The heterosis calculated for this trait was observed to exhibit positive values varying between 84.9% (KA × CSR_2) to 155.5% (SR_1 × KA) (Table 3). Out of 66 hybrid combinations, 21 hybrids were found to exhibit significant ($P < 0.05$) better parent heterosis for this trait (Table 4).

Cocoon shell ratio (%): The mean values of the hybrids revealed variability to a great extent with a minimum of 20.4% (KA x NB_4D_2) to a maximum of 24.5% (CSR_2 x NB_4D_2). Out of 66 hybrids studied, 31 hybrids exhibited higher values above the over all mean of 0.38g (Table 2). The heterosis value calculated for this trait indicates positive values varying between 8.7 in KA x CSR_2 to 10.7% CSR_2 x NB_4D_2 (Table 3). However, none of the 66 hybrid combinations exhibited significant ($P < 0.05$) better parent heterosis for this trait.

Cocoon filament length (m): The mean values of the 66 hybrids revealed variability to a great extent of 728 m (KA x CSR_2) and a maximum of 1177 m (SR_1 x SR_4). Out of 66 hybrids studied, 32 hybrids have exhibited higher value over the mean of 549 (Table 2). The heterosis calculated for this trait was observed to exhibit positive values varying between 4.75 (CSR_4 x CSR_{19}) to 52.89% (CSR_2 x NB_4D_2) presented in Table 3. Out of 66 hybrid combinations 51 hybrids were found to exhibit significant ($P < 0.05$) better parent heterosis for this trait (Table 4).

Raw silk (%): The mean values of 66 hybrids revealed variability to a extent with a range of 11.7% (KA x NB_4D_2) to 18.4% (SR_3 x SR_5). Out of 66 hybrids studied, 36 hybrids have shown higher mean values than the over

Table 5. Anova of 8 economic traits of pure lines and their hybrids

		Mean sum of squares							
Source of variation	df	Fecundity	Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio	Filament length	Raw silk
Replicates	2	213.55128	6.05556	0.50355*	0.00168	0.00036**	1.66324	261.81197	0.67269**
Treatments	77	1378.50067***	450.83544***	18.0824***	0.031241***	0.00169***	4.73448***	29621.72389***	6.70833***
Parents	11	764.73485**	854.20202***	18.46872***	0.02509***	0.00143***	4.73572**	19872.00758***	4.59716***
Hybrids	65	1335.34079***	346.40132***	13.52315***	0.02444***	0.00131**	4.80103***	21420.59736***	4.61656***
Parents vs hybrids	1	10935.31643***	2802.02096***	310.18975***	0.54088***	0.02907***	0.39546	669941.82770***	165.89642***
Error	154	290.11838	5.39755 1	0.1564	0.00090	0.00007	1.82580	361.01110	0.11570

*Significant ($P < 0.05$), **Significant ($P < 0.01$), N.S : Non Significant, ***$P < 0.01$.

all mean of 16.3% (Table 2). The heterosis calculated for raw silk percent trait was observed to exhibit positive values varying between 4.1 to 52.1% (Table 3). Out of 66 hybrid combinations, majority of hybrids were found to exhibit significant ($P < 0.05$) better parent heterosis values for this trait.

Overall results indicates that, the hybrids $SR_1 \times SR_4$, $SR_3 \times SR_6$, $SR_2 \times CSR_4$, $SR_2 \times SR_5$, $CSR_2 \times NB_4D_2$, $CSR_2 \times CSR_4$ and $SR_4 \times NB_4D_2$ have expressed significant mid parent values for seven quantitative traits out of eight traits evaluated. Similarly, the hybrids $SR_1 \times SR_5$, $CSR_2 \times SR_5$, $SR_4 \times CSR_{19}$, $SR_4 \times CSR_4$ have expressed significant better parent values for six traits out of eight traits evaluated indicates their capacity to yield more silk under tropical areas.

Analysis of variance results reveal great deal of variability (Table 5) indicating significant variations ($P < 0.001$) between treatments, parents, hybrids and parents Vs hybrids. However significant variations were observed ($P < 0.01$) for cocoon yield, cocoon shell weight and raw silk among the replicates and variations were found to be non-significant for the ($P > 0.01$) remaining traits.

Discussion

In India, indigenous races are well adapted to fluctuating tropical climatic conditions, but they are poor in productivity and efforts are made over a decade to improve the quality of raw silk through the introduction of more flexible superior bivoltine hybrids. In the present investigation, the high variability noticed for all important economic traits in the hybrids can be attributed to genetic diversity present among the lines utilized in hybrid preparation. The newly evolved lines showing differences among themselves can be related to the genetic mechanism developed in response to different intensity of selection pressure followed during the course of breeding. As a result, different gene frequencies for various characters appear to posses its specific genetic constitutions and ability to transmit the same to their offspring. Heterosis is the function of dominance effect and genetic distance between parents. When two completely genetically diverge homozygous parents are crossed maximum heterozygosity can be achieved and this in turn leads to significant heterosis (Falconer, 1981).

This phenomenon can be attributed to the genetic interaction, additive gene action, dominance, penetrance and expressivity of genes in question which play an important role in the expression of hybrid vigour. The phenomenon of heterosis with regard to each of the hybrids derived from the lines is an important aspect which enables to understand the manifestation of hybrid vigour in respect of each of the characters independently and in conjugation with others. In order to obtain desirable economic effects, skillful utilization of heterosis is an important step and the present findings are in agreement with Basavaraja (1996).

Among the lines SR_1, SR_3 and SR_6 performed well for majority of the traits evaluated and majority of the newly evolved thermo-tolerant hybrids exhibited significant mid parent heterotic values (> 10). Similarly, better parent heterotic values (> 6) for important traits like pupation rate, cocoon yield, cocoon weight and filament length showing complete dominance indicates the influence of additive genes. Similar observations were also made by Bhargava *et al.* (1996) and Ramesh Babu *et al.* (2001) in different crosses of silkworm. Parents with higher gca values are expected to produce high heterosis as gca consists of additive gene effects and additive × additive type of interactions which are heritable. The presence of highly significant better parent heterotic effects for cocoon weight in four hybrids which is having positive correlation to cocoon yield may increase overall silk productivity is in concurrence with Subba Rao *et al.* (1990). Thus the present study is having significant value in identifying the best flexible bivoltine hybrids ($SR_1 \times SR_5$ and $CSR_2 \times SR_5$) suitable for tropical climates for commercial exploitation.

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