

Line × Tester Analysis of Certain Quantitative Traits in Silkworm *Bombyx mori* L. under Optimum and Stress Rearing Conditions

P. Sudhakara Rao^{1*}, R. K. Datta², K. M. Vijaya Kumari¹, A. K. Palit¹ and S. A. Bhat¹

¹Central Sericultural Research and Training Institute, Mysore - 570 008, India.

²Director (Retired-CSRTI) No:202, 8th cross, Srirampura II stage, Mysore - 23. India.

(Received 30 August 2003; Accepted 3 December 2003)

The combining abilities in the 5 newly evolved thermo tolerant breeds viz., SR6, SR7, SR8 SR9 and SR10 of silkworm *Bombyx mori* L. and their 15 hybrids were made in a line × tester crossing programme. Data were analysed for seven quantitative traits i.e., pupation rate, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length and raw silk percentage under optimum room temperature ($25 \pm 1^\circ\text{C}$) conditions (In case of high temperature ($36 \pm 1^\circ\text{C}$) stress conditions five economic traits except filament length and raw silk percentage) with 3 widely adapted testers i.e., KA, CSR2 and CC1 as lines (females) and testers (males) respectively. The performance at high temperature and low humidity conditions are only taken into consideration for selecting the best lines/hybrids. Among the lines SR6 exhibited positive General combining ability (GCA) effects for pupation rate, cocoon yield, cocoon weight and cocoon shell ratio traits, followed by SR7 for pupation rate, cocoon yield and cocoon shell weight and cocoon shell ratio. Among testers, KA exhibited positive GCA effects for two quantitative traits cocoon yield, cocoon weight and CSR2 for cocoon shell weight and cocoon shell ratio under adverse temperature conditions. The hybrid SR6 × CC1 and SR7 × CSR2 exhibited significant positive Specific combining ability (SCA) effects for majority of the traits in high temperature stress conditions of rearing. The better parent value of heterosis (Heterobeltiosis) was exhibited by the hybrid SR6 × CC1 for pupation rate, cocoon yield, cocoon weight and cocoon shell weight and SR7 × CSR2 for all the traits evaluated under high temperature conditions.

Based on the results, the lines SR6 and SR7 was judged as best combiners and the hybrids SR6 × CC1 and SR7 × CSR2 can be selected for commercial exploitation in tropical climate.

Key words: *Bombyx mori* L., Combining ability, Temperature and humidity, Hybrids

Introduction

Combining ability is the most widely used biometrical tool in determining the genetic worth of the parents/hybrids in detecting the magnitude of variability present in the organism (Arunachalam, 1994). The phenomenon of combining ability has been extensively studied in silkworm *Bombyx mori* L. to identify promising hybrids (Gamo and Hirabayashi, 1983; Kantaratnakul *et al.*, 1987; Datta and Pershad, 1988; Tayade, 1989; Singh *et al.*, 2000; Datta *et al.*, 2001; Sudhakara Rao *et al.*, 2001) under favourable environment. But only few reports are available on analysis of heterosis and combining ability under stress conditions (Malik *et al.*, 1999; Suresh Kumar *et al.*, 1999). The major problem of tropical sericulture is need of more flexible hybrid combinations with genetic plasticity to buffer the tropical conditions like high temperature and low humidity ($36 \pm 1^\circ\text{C}$ and $50 \pm 5\%$). The genotypes which fared well under optimum temperature and humidity conditions are unable to with stand adverse climatic conditions of the tropics (Datta *et al.*, 1997) particularly during summer months and the summer cocoon crop is very important for small and marginal farmers. Keeping in view of this, an attempt has been made to assess and analyse heterosis and combining ability under optimum room temperature and high temperature and low humidity conditions to identify most promising hybrid(s) from the newly evolved thermo-tolerant breeds. These

*To whom correspondence should be addressed.

Central Sericultural Research and Training Institute, Mysore - 570 008, India. Tel: +091-821-2362571; Fax: +091-821-362845; E-mail: parimi_rao@yahoo.com

lines are evolved for high temperature and low humidity ($36 \pm 1^\circ\text{C}$ and $50 \pm 5\%$) conditions using polyvoltine blood and are characterized by higher survival and productivity merits.

Materials and Methods

Five newly evolved thermo-tolerant bivoltine breeds *viz.*, SR6, SR7, SR8 (marked larvae) and SR9 and SR10 (with plain larvae) spinning white dumb-bell cocoons are crossed with popular bivoltine breeds *i.e.*, KA, CSR2 and CC1 spinning white oval cocoons as lines (female) and testers (male) respectively to raise 15 hybrids. The silkworm rearing was carried out following the standard method (Krishnaswami, 1978) till second day of fifth instar. Four hundred larvae per bed were counted and retained after third moult. On the completion of 48 hrs of 5th instar, which is appropriate time for exposure to high temperature (Tazima and Ohnuma, 1995), 100 larvae were separated from each bed for the thermal treatment of high temperature ($36 \pm 1^\circ\text{C}$) with low humidity ($50 \pm 5\%$ - T1) in sericatron (an environmental chamber having precise mechanism to control temperature and humidity at desired level). The remaining larvae were served as control batch under optimum room temperature ($25 \pm 1^\circ\text{C}$; Relative humidity $70 \pm 5\%$ - T0). Rearing was conducted during Jan - Feb 2002 in three replications in each treatment. Larvae were subjected to high temperature conditions 6 hrs daily (10 am to 4 pm) and afterwards shifted to ambient room temperature till cocooning (Kato, 1989). Data were recorded for seven quantitative traits of economic importance *viz.*, pupation rate, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio percentage, filament length and raw silk percentage (In case of high temperature batches, only 5 traits were considered except filament length and raw silk percentage).

The data pertaining to general and specific combining

ability of the lines, testers and hybrids were estimated by employing the statistical method of Kempthorne (1957). The data pertaining to the expression of Heterosis and heterobeltiosis is calculated by employing the following formulae by keeping the check hybrid KA \times NB₄D₂ in all treatments (Harada, 1961).

$$\text{Heterosis (\%)} = \frac{\text{Mean of the hybrid (F}_1\text{)} - \text{Mid-parent value (MPV)} \times 100}{\text{Mid-parent value (MPV)}}$$

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

Where,

H = Percentage of heterosis

F_1 = Mean value of the hybrid

MP = Mean value of the mid parent.

BP = Mean value of the better parent

Results

Analysis of variance

Analysis of variance of combining ability for lines, testers and Line \times Testers are presented in Table 1 and 2 for T0, 3 and 4 for T1. Significant differences were observed among the lines for all the traits. The large mean sum of squares values noticed for pupation rate, cocoon yield, cocoon weight and filament length indicates more variability of the lines under T0 (Table 1). Favorable climate is helped them to express equally for all traits in testers and line \times testers are shown almost equal performance under in T0. Where as narrow differences were noticed in T1 for pupation rate and filament length (Table 2). The narrow mean sum of squares for lines compared to that of testers and line \times testers in high temperature indicated more diversity for combining ability in lines and their well

Table 1. Analysis of variance of combining ability and estimates of variance components for certain quantitative traits in *Bombyx mori* L. under room temperature ($25 \pm 1^\circ\text{C}$, relative humidity $70 \pm 5\%$)

Source of Variation	D. f.	Mean sum of squares						
		Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio	Filament length	Raw silk
Lines	4	1074.20	24.354	332.12	51.885	10.214	1065.111	7.760
Testers	2	131.4888	3.484	217.934	15.284	11.599	5948.34	3.094
Line \times Testers	8	354.433**	7.847**	218.834**	11.561	3.476	20433.4**	3.275**
Error	28	4.884	0.119	7.685	0.8677	0.4978	1655.246	0.3047

*Significant at 5% level and **significant at 1% level.

Table 2. Analysis of variance of combining ability and estimates of variance components for certain quantitative traits in *Bombyx mori* L. under High temperature ($36 \pm 1^\circ\text{C}$) and low humidity ($50 \pm 5\%$) conditions

Source of Variation	D. f.	Mean sum of squares				
		Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio
Lines	4	385.388	14.812	370.589	18.252	9.740*
Testers	2	69.622	7.521	357.840	15.428	4.399
Line × Testers	8	184.622**	5.958**	124.306**	6.55**	1.901**
Error	28	3.1317	0.186	7.987	0.420	0.191

*Significant at 5% level and **significant at 1% level.

Table 3. Estimates of general combining ability of parents for certain quantitative traits in *Bombyx mori* L. under room temperature ($25 \pm 1^\circ\text{C}$, relative humidity $70 \pm 5\%$)

Lines	Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio	Filament length	Raw silk
SR6	9.400**	1.135**	-1.640	-1.122	-0.594	-30.308	-0.862**
SR7	-1.156	0.079	5.438**	3.456	1.690	-17.784	1.498**
SR8	-12.156**	-1.983**	-7.173**	-2.933	-1.085	21.106	0.242
SR9	12.733**	2.013**	7.082**	1.033	-0.285	34.472	-0.337
SR10	-8.822**	1.243**	-3.707*	-0.433	0.273	-43.054	-0.541
Testers							
KA	2.244	0.515*	3.774*	-0.462	0.318	-11.893	-0.413
CSR2	-3.356*	-0.440*	0.131	1.158	0.640	-11.097	0.486
CC1	1.111	-0.075	-3.876*	-0.696	-0.958	22.990	-0.073

*Significant at 5% level and **significant at 1% level.

Table 4. Estimates of general combining ability of parents for certain quantitative traits in *Bombyx mori* L. under high temperature ($36 \pm 1^\circ\text{C}$) and low humidity ($50 \pm 5\%$)

Lines	Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio
SR6	2.222*	0.883**	7.251**	0.549	0.664*
SR7	5.889**	0.756**	-1.404	1.982**	1.450**
SR8	2.222**	0.826**	6.062**	-0.218	-0.968**
SR9	-11.222**	-2.102**	-7.071**	-0.396	0.736**
SR10	0.889	-0.363	-4.838**	-1.918**	-0.555
Tester					
KA	1.882	0.810*	4.998**	0.287	-0.506*
CSR2	-2.378*	-0.308	-0.236	0.840*	0.571*
CC1	0.556	-0.502*	-4.672*	-1.127*	-0.065

*Significant at 5% level and **significant at 1% level.

adaptation to high temperature conditions.

General combining ability (GCA) effects

General combining ability (GCA) effects in five lines and three testers with respect to seven quantitative characters (in case of T1 five quantitative characters) are presented in Table 3 and 4.

Among the lines SR 6 and SR7 exhibited positive GCA effects for four traits. The line SR6 shown positive GCA effects for pupation rate, cocoon yield, cocoon weight and

cocoon shell ratio followed by SR7 for pupation rate, cocoon yield, cocoon shell weight and cocoon shell ratio (Table 4). Among the testers, KA has exhibited significant GCA effects for cocoon yield and cocoon weight and CSR2 for cocoon shell weight and cocoon shell ratio under T1 conditions.

Specific combining ability (SCA) effects

Specific combining ability of top two hybrids under optimum and high temperature conditions is presented in

Table 5. The hybrid SR6 × CC1 and SR7 × CSR2 has shown to possess positive SCA effects under the adverse temperature and humidity conditions for majority of the traits evaluated (T1). The hybrid SR6 × CC1 has shown positive SCA effects for pupation rate, cocoon yield and cocoon weight; whereas SR7 × CSR2 has shown positive effects for cocoon yield, cocoon shell weight and cocoon shell ratio out of 5 traits evaluated.

Heterosis and heterobeltiosis

The heterosis and heterobeltiosis of top two selected hybrids are presented in Table 6. The hybrid SR6 × CC1 exhibited heterobeltiosis for four quantitative traits out of five traits evaluated over check hybrid KA × NB₄D₂ *i.e.*, pupation rate, cocoon yield, cocoon weight and cocoon shell weight. The hybrid SR7 × CSR2 has significant heterobeltiosis for all economic traits *viz.*, pupation rate, cocoon yield, cocoon weight, cocoon shell weight and cocoon shell ratio under T1 conditions.

Discussion

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. The improvement obtained by selection under favourable conditions will not help in realising the full potential when the selected strains are transferred to unfavourable conditions (Falconer, 1960). In the present study, heterosis, GCA of the lines and testers and SCA of the hybrids are analysed under optimum temperature and high temperature conditions to identify the best combiners and short-listing promising hybrids. It is evident from the Table 1, 2, 3, 4, 5 and 6 that the lines, testers and hybrids are performed well under optimum rearing conditions, but when they are subjected to adverse climatic conditions their performance was badly affected. Among the lines SR6, SR7 and SR9 performed well for majority of the traits evaluated under

optimum rearing conditions. For example the line SR9 expressed positive GCA effects for majority of the traits under T0 condition (Table 1) is unable to withstand for high temperature. Similarly the hybrids SR6 × CC1, SR7 × CC1 and SR10 × KA performed well under optimum temperature (T0) and shown significant positive SCA effects; whereas in adverse climatic conditions (T1) they are badly affected except SR6 × CC1. The lines SR6 and SR7 exhibited highly significant GCA effects for majority of the traits in adverse climatic conditions (Table 4) confirms their superiority. Parents with higher GCA values produce high heterosis as GCA consists of additive gene effects and additive × additive type of interactions, which are heritable. The hybrids SR6 × CC1 and SR7 × CSR2 × have shown positive SCA effects in adverse climatic conditions (T1) for majority of the quantitative traits evaluated (Table 5) indicates their superiority. Kato *et al.*, (1989) conducted series of experiments and observed that the resistance to high temperature is a dominant and heritable character. Specific combining ability (SCA) consists of non-additive effects, dominant effects and other interactions. This may be due to the fact that where these two thermo-tolerant female parents (SR6 and SR7) are involved in the crosses, their beneficial components might have expressed in hybrids. Suresh Kumar *et al.*, (2001) observed that there is maternal inheritance effect regarding temperature tolerance as evident from the better performance of those hybrids where the female parent used was more tolerant as pure breed. It was also noted that in all the hybrid combinations there was no maternal effect under normal rearing conditions and it was observed only in high temperature treated batches. It was interesting to note that during favourable season, non-additive genetic variance was important; whereas additive genetic variance was important during unfavourable season for the inheritance of cocoon breadth (Rahman and Jahan, 1997). This may be also true for the other traits like cocooning yield and cocoon weight as noticed in the present study. The better performance of SR6 × CC1 and SR7 × CSR2

Table 5. Specific combining ability effects of selected top two hybrids for certain quantitative traits in *Bombyx mori* L. under optimum and high temperature conditions

Crosses	Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio	Filament length	Raw silk
Room temperature (25 ± 1°C) and relative humidity (70 ± 5%)							
SR6 × CC1	12.667**	1.961**	6.620**	2.462	0.819	-21.290	1.112**
SR10 × KA	11.089**	1.881**	8.000*	2.140	0.318	45.686	0.208
High temperature (36 ± 1°C) and low humidity (50 ± 5%)							
SR6 × CC1	7.111**	1.341**	4.829**	0.604	-0.265	---	----
SR7 × CSR2	1.378	0.881**	2.624	2.271**	1.036**	----	----

*Significant at 5% level and **significant at 1% level.

Table 6. Heterobeltiosis effects of selected top two hybrids for certain quantitative traits in *Bombyx mori* L. under optimum and high temperature conditions

Crosses	Pupation rate	Cocoon yield	Cocoon weight	Shell weight	Shell ratio	Filament length	Raw silk
Room temperature (25 ± 1°C) and relative humidity (70 ± 5%)							
SR6 × CC1	29.79**	35.61**	3.64**	7.82**	-1.14	2.39	7.556**
SR10 × KA	-0.2.08	-34.12**	-7.30**	-5.79	12.51**	-0.51	4.73*
High temperature (36 ± 1°C) and low humidity (50 ± 5%)							
SR6 × CC1	23.15**	32.09**	9.79**	11.23**	1.30	---	----
SR7 × CSR 2	13.06**	30.85**	6.61*	23.40**	15.77**	---	----

*Significant at 5% level and **significant at 1% level.

hybrids under high temperature and low humidity condition is in concurrence with earlier studies of Nagaraju *et al.* (1996) wherein it was emphasized that the performance of their hybrids will be much superior to both the parents when both the parental strains are raised under unfavourable conditions. The hybrids SR6 × CC1 and SR10 × KA has expressed positive better parent value of heterosis (Heterobeltiosis) over check hybrid KA × NB₄D₂, but SR10 × KA hybrid has poorly performed under stress rearing conditions. Only SR6 × CC1 expressed positive heterobeltiosis for four quantitative traits and SR7 × CSR2 for all the five traits evaluated.

The results of the present study indicate that the expression of hybrid vigour is different for hybrids at different temperature treatments. It is clear that the level of heterosis present in the hybrids can be influenced by the environmental factors and the findings are in agreement with Suresh Kumar *et al.* (1999). Based on the results, it is concluded that the newly evolved lines SR6 and SR7 are good general combiners and the hybrids SR6 × CC1 and SR7 × CSR 2 can be exploited commercially in adverse climatic conditions prevailing in the tropics.

Acknowledgement

The first author wishes to express his gratitude to Dr. S. B. Dandin, Director of the institute for providing laboratory facilities for the study and Dr. B. Nataraju, Deputy Director for his encouragement.

References

- Arunachalam, V. (1994) The fallacy behind the use of a modified line × tester design. *Indian J. Genet. Plant. Breed.* **34**, 280-287.
- Datta, R. K. and G. D. Pershad (1988) Combining ability among multivoltine × bivoltine silkworm (*Bombyx mori* L.) hybrids. *Sericologia* **28**, 21-29.
- Datta, R. K., H. K. Basavaraja, N. Suresh Kumar, C. M. Kishore Kumar and S. Nirmal Kumar (1997) Evolution of robust hybrids of silkworm (*Bombyx mori* L.), for tropics. XVIIth Congr. of Int. Seric. commi. 22-26 April, Brazil.
- Datta, R. K., D. Raghavendra Rao, K. P. Jayaswal, V. Premalatha, Ravindra Singh and B. K. Kariappa (2001) Heterosis in relation to combining ability in multivoltine and bivoltine strains of silkworm, *Bombyx mori* L. *Indian. J. Seric.* **40**, 1-6.
- Falconer, D. S. (1960) Selection in mice for growth on high and low planes of nutrition. *Genet. Res.* **1**, 91-113.
- Gamo, T. and T. Hirabayashi (1983) Genetic analysis of growth rate, pupation rate and some quantitative characters by diallel cross in silkworm, *Bombyx mori* L. *Jpn. J. Breed.* **33**, 178-190.
- Harada, C. (1961) On the heterosis of quantitative characters in the silkworm. *Bull. Seri. Expt. Stn.* **17**, 50-52.
- Karantaratakul, S., Tharovarnakulkit, S. Wongthong, A. Aronying, Compiranon and P. Saksoong (1987) Diallel crosses with polyvoltine strains of Thai silkworm *Bombyx mori* L. *Sericologia* **27**, 279-286.
- Kato, M., K. Nagayasu, O. Ninaki, W. Hara and A. Watanabe (1989) Study on resistance of the silkworm, *Bombyx mori* to high temperature. *Proc. Int. Congr. SABRAO.* **2**, 953-956.
- Kempthorne, O. (1957) An introduction to Genetic statistics, John Wiley & Sons, London.
- Krishnaswami, S. (1978) New technology of silkworm rearing. *Bull. C. S. R. & T. I.* **2**, 1-24.
- Malik, G. N., M. A. Masoodi, A. S. Kamili and M. Aijaz (1999) Combining ability analysis over environments in diallel crosses of bivoltine silkworm *Bombyx mori* L. *Indian. J. Seric.* **38**, 22-25.
- Nagaraju, J., S. Rajeurs and R. K. Datta (1996) Cross breeding and heterosis in the silkworm, *Bombyx mori*. A review. *Sericologia* **36**, 1-20.
- Rahman, S. M. and M. S. Jahan (1997) Heterosis and combining ability estimate for cocoon breadth in silkworm, *Bombyx mori* L. *Bull. Seric. Res.* **8**, 7-11.
- Singh, R., G. V. Kalpana, P. Sudhakara Rao, M. M. Ahsan, R. K. Datta and M. Rekha (2000) Studies on combining ability and heterosis in the silkworm, *Bombyx mori* L. *Indian. J. Seric.* **39**, 43-48.

- Sudhakara Rao, P., H. K. Basavaraja, V. Nishitha Naik, K. P. Jayaswal, M. Rekha and R. K. Datta (2001) Evaluation of combining ability in hybrids between polyvoltine and bivoltine sex-limited cocoon colour breeds of silkworm, *Bombyx mori* L. *Indian. J. Seric.* **40**, 163-167.
- Suresh Kumar, N., C. M. Kishore Kumar, H. K. Basavaraja, N. Mal Reddy, M. Ramesh Babu and R. K. Datta (1999) Comparative performance of robust and productive bivoltine hybrids of silkworm, *Bombyx mori* L., under high temperature conditions. *Sericologia* **39**, 567-571.
- Suresh Kumar, N., T. Yamamoto, H. K. Basavaraja and R. K. Datta (2001) Studies on the effect of high temperature on F1 hybrids between Polyvoltine and bivoltinesilkworm races of *Bombyx mori* L. *Int. J. Indust. Entomol.* **2**, 123-127.
- Tayade, D. S. (1989) Genetic architecture of economic traits in strains of mulberry silkworm, *Bombyx mori* L. 1. Combining ability analysis. *Sericologia* **29**, 43-60.
- Tazima, Y. and A. Ohnuma (1995) Preliminary experiments on the breeding procedure for synthesizing a high temperature resistant commercial strain of the silkworm, *Bombyx mori* L. *Silk. Sci. Res. Inst. Jpn* **43**, 1-16.