Int. J. Indust. Entomol. Vol. 13, No. 2, 2006, pp. 73~77

International Journal of
Industrial Entomology

Estimation of Heterosis and Combining Ability in Hybrids between Resistant and Susceptible Bivoltine Breeds of Silkworm *Bombyx mori* to Densonucleosis Virus1 (BmDNV1)

P. Sudhakara Rao*, B. Nataraju, M. Balavenkatasubbaiah and S. B. Dandin
Central Sericultural Research and Training Institute, Mysore - 570 008, India

(Received 15 June 2006; Accepted 17 November 2006)

Combining ability and hybrid vigour analysis was carried out in hybrids between newly developed non-susceptible lines to BmDNV1 and popular bivoltine breeds for certain quantitative traits *viz*. Pupation rate, Cocoon yield, Cocoon weight, Cocoon shell weight and Cocoon shell ratio, Survival rate against BmIFV and BmNPV. General combining ability (GCA) effects revealed that among the lines CSR2DR was found good general combiner exhibiting significant GCA effects for six characters, out of seven traits evaluated. Among testers CSR28DR was found as good combiner exhibiting significant GCA effects for six traits. Out of 36 hybrids made between resistant × resistant, resistant × susceptible and susceptible × susceptible breeds, one hybrid CSR21DR × CSR28DR exhibited significant SCA effects for six traits. The selected hybrid CSR21DR × CSR28DR also exhibited significant positive heterosis and heterobeltiosis expressions for maximum traits and could be exploited as commercial silkworm hybrid resistant to important viral diseases.

Key words: *Bombyx mori*, bivoltine hybrids, BmDNV1, combining ability, heterosis

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 (Yokoyama, 1957; Hirobe, 1968). 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 don't add much value. Variation in the genetic architecture and the degree of genetic divergence between the concerned pure lines, the study of their F_1 performance have significance relevance. The genetic worth of the pure lines can be determined by evaluating their general and specific combining abilities (Sprague and Tatum, 1942). The critical assessment of variability present in the breeding material through combining ability studies is necessary in giving way for combining most of the desirable traits available in different genotypes into a single breed/hybrid and identify best hybrid combinations. Conventional breeding methods are directed not only to synthesize new breeds but also their selection in the cross breeding programme to identify the promising hybrid combinations for commercial exploitation. It is essential therefore to evaluate the genetic worth of the pure breeds and their general and specific combining ability of the hybrids to understand the magnitude of heterosis (Gamo, 1976; Hirobe, 1985).

The knowledge of biometrical genetics has helped to develop several methods of which diallel cross technique and Line × Tester analysis have been extensively used in plants and animals to evaluate the combining ability of pure lines and to identify superior hybrid (Kempthorne 1957).

Frequent crop losses in sericulture particularly in tropical areas can be attributed to aggravated silkworm diseases coupled with unfavorable weather conditions. Although the disinfection of rearing place and apparatus are carried out before onset of silkworm rearing, it is not necessarily adequate to prevent the occurrence of silk-

---

*To whom the correspondence addressed
Central Sericultural Research and Training Institute, Mysore - 570 008, India. Tel: +91-821-2900571; Fax: +91-821-2362845; E-mail: parimi_rao@yahoo.com

worm diseases. In view of inadequate disinfection and unhygienic conditions in silkworm rearing place, use of commercial silkworm breeds resistant to important silkworm diseases is better option (Eguchi *et al.*, 1998). Keeping this in mind, a breeding programme was initiated at this institute during the year 2000 and evolved 6 productive bivoltine breeds non-susceptible to BmDNV1. In the present investigation, an attempt has been made to evaluate the combining ability of the six newly developed lines non-susceptible to *Bombyx mori* Densonucleosis Virus type1 (BmDNV1) CSR2DR, CSR21DR, CSR23DR, CSR4DR, CSR5DR and CSR28DR. Six popular productive bivoltine breeds, but susceptible CSR2, S1, S5, CSR6, D13 and CSR16 were used in the study to identify the best combiners and short-listing promising hybrids for imperfect disinfection conditions.

Materials and Methods

The females of three newly developed lines non-susceptible to *Bombyx mori* Densonucleosis Virus type1 (BmDNV1) *viz.* CSR2DR, CSR21DR and CSR23DR and productive bivoltine breeds but susceptible to BmDNV1, CSR2, S1 and S5 (spinning oval cocoons) were used as lines. Similarly, CSR4DR, CSR5DR and CSR28DR non-susceptible to BmDNV1 and popular dumbbell breeds, but susceptible to BmDNV1, CSR6, D13, CSR16 (Spinning dumbbell cocoons) are used as testers. 36 crosses were made between resistant × resistant, resistant × susceptible and susceptible × susceptible breeds. General and specific combining abilities were determined by employing the Line × Tester technique out lined by Kempthorne (1957). All the 36 hybrids along with their parents were reared in three mass replicates. After passing second moult 300 larvae per each replication were taken and inoculated (P*eros* inoculation) with 10^{-2} concentration of BmDNV1 and continued till spinning. In another set, same population were inoculated with $10^{4.5}$ dilutions of BmIFV stock inoculum and 10^{7} POB/ml BmNPV and observed for 15 days of post inoculation in separate rooms. The control batches having same number of larvae were maintained in normal room conditions without any inoculation. Data were collected on five economic traits such as pupation rate, cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio for BmDNV1 inoculated batches and survival rate against two viral pathogens of the silkworm BmIFV and BmNPV. Since the objective of the study is to identify potential hybrids resistant to Viral flacherie disease , performance of the inoculated rearing batches were only taken in to consideration for statistical analysis. The data pertaining to the expression of Heterosis and heterobeltiosis in hybrids with regard to above mentioned five economic traits of importance and survival rate for two pathogens were studied by employing the formulae out lined by Harada, 1961.

Results

Analysis of variance of combining ability for lines, testers and line × testers is presented in Table 1. Significant differences were observed among the lines for all the characters studied except for cocoon shell ratio. Similarly testers have shown significant differences for all the traits except for pupation rate. Line × testers exhibited maximum contribution for all the traits evaluated.

General combining ability (GCA) effects in the lines and testers with respect to five quantitative traits and survival percent against two diseases is presented in Table 2. Among the lines, the newly developed line CSR2DR exhibited significant GCA effects for all the characters except cocoon yield/10,000 larvae by weight. Among the testers, CSR28DR exhibited positive GCA effects for all the traits except for cocoon yield.

Specific combining ability (SCA) effects for the selected hybrids are presented in Table 3. Among the hybrids, one hybrid CSR21DR × CSR28DR excelled in all the traits except for cocoon shell ratio; followed by four other hybrids CSR2DR × CSR4DR, CSR2 × CSR16, S1 ×

Table 1. Analysis of variance of combining ability and estimates of variance for certain quantitative traits

Source of variation	Df	Mean sum of squares						
		Pupation BmDNV1)	Yield 10,000 L	Cocoon weight	Shell weight	Shell ratio	Survival on inoculation (%)	
							BmIFV	Bm NPV
Lines	5	16.554***	1.633***	0.006***	0.0002**	0.155	3023.156***	1373.289***
Testers	5	3.714	0.693*	0.010***	0.002***	1.051*	110.250**	1667.361***
Line × Testers	25	60.791***	2.476***	0.011***	0.001***	1.643***	504.360***	473.449***
Error	94	2.492	0.249	0.001	0.000	0.188	10.064	15.698

*Significant 5%, **Significant 1%

Table 2. Estimates of general combining ability (GCA) of quantitative traits and resistance

Parents	Pupation (BmDNV)	Yield 10,000 L	Cocoon weight	Shell weight	Shell ratio weight	Percent survival on inoculation	
						BmIFV	BmNPV
LINES							
CSR2DR	3.394***	0.169	0.007*	0.004**	0.122*	18.310***	3.611**
16DR	0.505*	-0.466***	-0.063***	-0.018***	-0.455***	30.505***	5.056***
18DR	-8.356***	0.064	0.036***	0.013***	0.239**	15.560***	3.889***
CSR2	2.560***	0.456***	0.028***	0.007***	0.025	16.384***	-23.111***
S1	1.699***	0.060	-0.020***	-0.017***	-0.534***	-23.245***	5.389***
S5	0.199	-0.284**	0.001	0.011***	0.604	-24.745***	5.167***
CD at 5%	0.5857	0.2172	0.0072	0.0029	0.1422	1.5674	1.9765
TESTERS							
CSR4DR	-1.718***	0.348*	0.003	-0.002	-0.192*	15.644***	10.722***
CSR5DR	-0.579	0.056	0.001	0.004*	0.247*	16.449***	20.667***
CSR28DR	2.060***	-0.108	0.021***	0.011***	0.385**	12.421***	14.056***
CSR6	3.019***	-0.293*	-0.009*	-0.009*	-0.388***	-15.995***	-14.667***
D13	-2.468***	-0.136	-0.004	-0.004*	-0.180*	-14.384***	-13.167***
CSR16	-0.315	0.135	-0.012	0.001	0.128	-14.134***	-17.611***
CD at 5%	0.5857	0.2172	0.0072	0.0029	0.1422	1.5674	1.9765

*Significant 5%, ** Significant 1%

Table 3. Estimates of specific combining ability (SCA) effects for the selected hybrids under inoculated conditions

Hybrids	Pupation (BmDNV)	Yield 10,000L	Cocoon weight	Shell weight	Shell ratio	Percent survival on inoculation	
						BmIFV	BmNPV
CSR2DR × CSR4DR	3.634**	0.147	0.007	0.011*	0.544*	10.495***	7.667*
CSR21DR × CSR28DR	8.190***	0.918*	0.033**	0.015**	0.343	15.184***	10.056**
CSR2 × CSR16	1.176	1.094**	0.070***	0.029***	0.698**	6.856**	-19.167***
S1 × D13	4.162***	0.979**	0.036**	0.009*	-0.141	13.745***	-0.222
S5 × CSR6	1.315	0.742*	0.099***	0.033***	0.646**	6.301*	-5.944*
CD at 5%	1.4346	0.5320	0.0175	0.0072	0.3484	3.8393	4.8415

*Significant 5%, **Significant 1%

D13 and S5 × CSR6 for majority of the traits.

Heterotic and heterobeltiotic effects for the selected hybrids are presented in Table 4. Among the hybrids CSR21DR × CSR28DR and CSR2 × CSR16 were expressed positive heterosis over mid parent value for all the traits evaluated. Similarly, these two hybrids also expressed significant positive heterobeltiosis for all the evaluated traits indicating their superiority as promising hybrids.

Discussion

The hybrid evaluation has been widely followed in silkworm to find out the genetic worth of the hybrids. In the present investigation, analysis of variance for five economic traits and screening for two diseases of 12 parental breeds, 36 hybrids and parents vs. hybrids indicate a great deal of differences among themselves in the phenotypic expression of most of the characters reflecting genotypic variability (Table 1). Significant ($P < 0.05$) variations were observed between the parents, hybrids, parents vs. hybrids in almost all the traits reflects the presence of considerable degree of heterosis. The newly evolved lines are showing significant variations except for cocoon shell ratio can be related to the genetic mechanism developed in the course of breeding.

Among the lines, CSR2DR have shown significant ($P < 0.05$) GCA for six economic traits out of 7 traits evaluated indicates its superiority and ability to combine with most of the breeds, followed by CSR21DR for 5 traits (Table 2). These values facilitate in sorting out better parents with desirable genes for different component characters contributing to economic traits and productivity. Among the testers CSR28DR excelled for six traits out of seven traits evaluated. Significant GCA effects are related to additive gene effects of additive × additive interactions (Griffing, 1956). Identification of lines based on GCA effects corroborates with the findings of Krishnaswami *et al.* (1964), Subba Rao (1983), Bhargava *et al.* (1992) Basavaraja (1996) and Nanjundaswami (1997).

Specific combining ability is another important aspect to be evaluated in the hybrids to estimate the superiority. As could be seen from the Table 3, the magnitude of SCA effects varies among hybrids. Among the hybrids CSR21DR × CSR28DR have shown positive SCA effects for six traits out of seven traits evaluated and reflect the allelic interaction with respect to these traits and is expected to give high heterotic response (Table 4). However, the other selected hybrids CSR2DR × CSR4DR, CSR2 × CSR16, S1 × D13 and S5 × CSR6 have positive SCA effects for five traits. The identification of hybrids based on SCA effects parallel the work of Kalpana (1992); Malik *et al.* (1998); Singh *et al.* (2001); Sudhakara Rao *et al.* (2001) and Ramesh Babu *et al.* (2003). The significant SCA values observed in these hybrids reflected the operation of non-additive gene effects in the expression of the traits. This clearly indicates that additive × dominance gene interactions were important in determining the SCA. Similar results were reported by Datta *et al.* (2001).

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 heterosis 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. In the present findings, the selected hybrids CSR21DR × CSR28DR and CSR2 × CSR16 have also shown significant heterosis and heterobeltiosis. The better performance of CSR21DR × CSR28DR hybrid under inoculated disease conditions is in concurrence with earlier studies of Nagaraju *et al.* (1996), wherein it was emphasized that the performance of their hybrids would be much superior to both the parents when both the parental strains are raised under unfavourable conditions. Therefore, the lines with positive GCA effects could be used for improvement of cocoon yield and best hybrid could be exploited as commercial silkworm hybrid resistant to important viral diseases.

Acknowledgement

The authors are thankful to Dr. H .K. Basavaraja, Joint Director of this institute for supplying silkworm breeds for the study and Mr. A .K. Palit, Assistant Director for statistical analysis.

Table 4. Estimates of Heterosis and heterobeltiosis in selected hybrids for certain quantitative traits under inoculated conditions

Crosses	Pupation (BmDNV)	Yield 10,000L	Cocoon weight	Shell weight	Shell ratio	Percent survival on inoculation	
						BmIFV	Bm NPV
CSR2DR × CSR4DR							
MPV	11.82**	14.19**	7.03**	10.32**	3.01*	-3.94	-5.70
BPV	9.83**	10.27**	4.90**	9.96**	1.26	-11.91**	-6.98
CSR21DR × CSR28DR							
MPV	5.70**	14.51**	10.09**	14.76**	4.17**	-2.44	7.72
BPV	4.43**	10.09**	7.53**	11.06**	3.02*	-3.23	3.20
CSR2 × CSR4DR							
MPV	7.14**	7.83**	3.56**	7.48**	3.78**	-42.30**	-32.37**
BPV	6.06**	6.52*	2.06*	4.40*	2.25	-68.23**	-54.65**
CSR2 × CSR16							
MPV	5.30**	13.45**	7.18**	12.48**	4.90**	38.18	-56.96**
BPV	4.92**	11.66**	7.00**	10.80**	3.16*	35.71	-61.36**
S5 × CSR6							
MPV	7.20**	9.86**	9.06**	13.45**	3.96**	-86.96**	-50.14**
BPV	6.61**	7.77*	4.50	10.14**	2.54	-88.46**	-62.23**

*Significant 5%, **Significant 1%

References

Basavaraja, H. K. (1996) Studies on the synthesis of productive bivoltine strains of *Bombyx mori* L. through hybridisation, selection and genetic evaluation. Ph.D Thesis, University of Mysore, Mysore.

Bhargava, S. K., V. Thiagarajan., M. Ramesh Babu, and B. Nagaraj (1992) Combining ability and genetic analysis of quantitative traits in silkworm (*Bombyx mori* L.) *J. Genet. & Breed.*, **46**, 327-330.

Datta, R. K., D. Raghavendra Rao, K. P. Jayaswal, V. Premalatha, Ravindrasingh 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.

Eguchi, R., W. Hara, A. Shimazaki, K., Hirota, K., M. Ichiba, O. Ninagi and K. Nagayasu (1998) Breeding of the silkworm race "Taisei" non-susceptible to a densonucleosis virus type 1. *J. Seric. Sci. Jpn.* **67**, 361-366.

Gamo, T. (1976) Recent concepts and trends in silkworm breeding. *Farming Japan* **10**, 11-22.

Griffing, B. (1956) Concepts on general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* **9**, 463-493.

Harada, C. (1961) On the heterosis of quantitative characters in Silkworm. *Bull. Seric. Expt. Stn.* **17**, 50-52.

Hirobe, T. (1968) Evolution, differentiation and breeding of the silkworm, the silk road. Past and present. In. *Genetics in Asian Countries XII International Cong. Genetics (Tokyo)*, 25-26.

Hirobe, T. (1985) On the recent advancement of silkworm breeding in Japan. *Tamagawa Univ.*, 2-17-19, *Gakuen-cho, Highshikurume-shi Jpn.*, 157-167.

Kalpana, G . V. (1992) Breeding of superior races of Silkworm *Bombyx mori* L. for tropical climates Ph.D Thesis, University of Mysore, Mysore. 288p.

Kempthorne, O. (1957) An Introduction to genetic statistics. *John Wiley and Sons Inc., Newyork.*

Krishnaswami, S., M. S. Jolly and S. Subba Rao (1964) Diallel analysis of quantitative characters in multivoltine races of silkworm . *Indian J. Genet. Pl. Breed.* **24**, 213-222.

Malik, G. N., M. A. Masoodi, A. S. Kamili and A. M. Sofi (1998) Studies on heterosis in some bivoltine silkworm (*Bombyx mori* L.) crosses. *J. Seric.* **6**, 47-49.

Nagaraju. J., S. Raje Urs and R. K. Datta (1996) Cross breeding and heterosis in the silkworm *Bombyx mori*, a review. *Sericologia* **36**, 1-20.

Nanjunda Swamy (1997) Studies on the combining ability in Silkworm *Bombyx mori* L. Ph.D thesis, University of Mysore, Mysore.

Ramesh Babu, M., Chandrashekharaiah, H. Lakshmi, A., K. Goel and J. Prasad (2003) Combining ability analysis of diallel crosses in bivoltine silkworm, *Bombyx mori* L. *Abstract in National Conference on Tropical Sericulture for Global Competitiveness 5-7 November* p.26.

Singh, R., D. Raghavendra Rao, B. K. Kariappa, V. Premalatha and S. B. Dandin (2003) Studies on analysis of combining ability in the mulberry silkworm *Bombyx mori* L. *Int. J. Indust. Entomol.* **6**, 107-113.

Sprague, G. F. and L. A. Tatum (1942) General vs specific combining ability in single crosses of corn. *J. Amer. Soc. Agron.* **34**, 923-932.

Subba Rao, G. (1983) Line × Tester analysis of some character in bivoltine silkworms, *Abst. Sem. Silk. Res. & Devpt.*, Bangalore, 15.

Sudhakara Rao, P., H. K. Basavaraja, V. Nishitha Naik, K. P. Jayaswal and M. Rekha (2001) Evaluation of combining ability in hybrids between polyvoltine × bivoltine (Sex-limited cocoon colour) breeds of silkworm *Bombyx mori* L. *Indian. J. Seric.* **40**, 163-16.

Yokoyama, T. (1957) On the application of heterosis in Japanese sericulture. *Proc. Int. Genet. Symp.*, 527-531.