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An Improved Method of Parthenogenetic Development and Analysis of Combining Ability in Bivoltine Breeds of the Silkworm, *Bombyx mori* L.

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Parthenogenesis, the development of unfertilized ovum opens new perspectives in silkworm breeding in the development of homozygous breeds. In order to improve induction of artificial parthenogenesis in the excised unfertilized eggs of different breeds of the silkworm, *Bombyx mori* L., a new method was devised and the results were compared with the routine method. General and specific combining abilities and hybrid vigour of newly developed bivoltine breeds were analyzed utilizing bivoltine breeds *viz.*, CSR_2, CSR_4, CSR_{17} and NB_4D_2. Estimation of GCA revealed superiority of the breeds, DNB_1 for eight characters followed by DNB_4 for five characters. Among the testers, CSR_2 was found good general combiner for seven characters followed by CSR_{17} for four characters. A great deal of variations was observed among the hybrids studied. Five hybrids namely, $DNB_1 \times CSR_2$, $DNB_4 \times CSR_4$, $DNB_4 \times NB_4D_2$, $DNB_6 \times CSR_2$ and $DNB_7 \times CSR_2$ showed significant SCA effects for 5-6 characters. The hybrid, $DNB_4 \times CSR_4$ showed its superiority by expressing significant hybrid vigour over BPV for 7 characters. Majority of the hybrids exhibited significant hybrid vigour for survival rate, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, filament length and denier.

Key words: Artificial partheogenesis, *Bombyx mori*, Combining ability, Heterosis, Hybrid vigour

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Introduction

Manifestation of heterosis in F_1 hybrids is the result of elimination of the action of harmful recessive genes with totally dominant genes separately inherited from both parents and favourable effects of some alleles in the heterozygous state (Strunnikov, 1986). With increased heterozygosity, hybrid vigour can be visualized when the offspring performs well above the average of their parents. Crossbreeding is extensively used in silkworm improvement as a means of exploiting heterosis (Nagaraju *et al.*, 1996). A knowledge of the extent and magnitude of heterosis helps in the isolation of superior segregates. Many intensive breeding efforts aiming at improving the productivity and quality of silk have yielded a number of inbred lines (Datta, 1984). Extensive studies have been carried out on the analysis of combining ability in the silkworm in order to select promising parents and hybrids (Satenahalli *et al.*, 1989; Subba Rao and Sahai, 1989; Rajalakshmi *et al.*, 1997; Rao *et al.*, 2002; Ravindra Singh *et al.*, 2000; 2001; 2003; 2005).

Parthenogenesis, the development of unfertilized ovum opens new perspectives in silkworm breeding in the development of homozygous silkworm breeds. Attempts to induce artificial parthenogenesis in the eggs of silkworm, *Bombyx mori* L. by means of diverse physicochemical stimuli such as various acids, alkali, electric stimuli, CO_2 and hot water have been reported (Strunnikov, 1983). Astaurov (1940) established a most suitable and reliable method of thermic parthenogenesis in the silkworm, *B. mori*. Significance of artificial parthenogenesis has been realized and its commercial utilization has well been demonstrated by many researchers (Strunnikov *et al.*, 1982; Strunnikov, 1986; Hirokawa, 1990; 1995; Takei *et al.*, 1990; Ravindra Singh *et al.*, 1994).

In the present study, artificial parthenogenesis coupled with conventional breeding has been utilized to judge the practical advantages of parthenogenesis like analysis of combining ability and heterosis in crosses involving newly developed bivoltine breeds as lines and popular bivoltine breeds as testers for various economic characters.

Materials and Methods

Six bivoltine breeding lines namely, DNB_1 and DNB_2 (Chinese oval type) and DNB_3, DNB_4, DNB_6 and DNB_7 (Japanese dumbbell type) developed through artificial parthenogenesis were utilized in the present study. Maintenance and multiplication of the silkworm breeds through artificial parthenogenesis were carried out initially following the routine method (46°C; 18 min) of Astaurov (1940). The rate of parthenogenesis percentage was found higher in F_1 hybrids. However, there was no improvement in the subsequent generations. Therefore, it was necessitated to improve the rate of parthenogenetic hatchability in the silkworm breeds by following a new improved method. A schematic representation of the routine and improved method has been shown in Fig. 1. Data were recorded for percentage of parthenogenetic development and hatching and comparison was made between the two methods to judge efficiency of the new method over the routine method. The ratio of reddish brown/dark pigmented eggs and total number of eggs treated was expressed as percentage of parthenogenetic development whereas the ratio of hatched larvae and number of pigmented eggs counted was considered as percentage of hatching. Hybrids were prepared using the bivoltine male parents of authorized CSR breeds of CSR_2, CSR_4, CSR_{17} and NB_4D_2. In each hybrid combination, three replicates were maintained.

Line × Tester analysis of Kempthorne (1957) was used to determine GCA effects of the lines and testers and SCA effects of the hybrids using following formulae:

General Combining Ability

a) Lines $gi = \frac{Xi}{tr} - \frac{X}{ltr}$, i=l to l

b) Testers $gj = \frac{Xj}{lr} - \frac{X}{ltr}$, j=l to t

Specific Combining Ability:

Hybrids $sij = \frac{Xij}{r} - \frac{Xi}{tr} - \frac{Xj}{lr} + \frac{X}{ltr}$

where, gi = general combining ability of lines
gj = general combining ability of testers
sij = specific combining ability of hybrids
l = number of lines
t = number of testers
r = number of replications
Xi = performance of l^{th} line with 't' testers
Xj = performance of j^{th} tester with 'l' lines
Xij = performance of (i × j) th hybrid and
X = grand total

Results

Induction of parthenogenetic development by means of improved and routine method in the excised unfertilized eggs of different silkworm breeds has been given in Fig. 2. When the eggs were artificially activated following the new method, the highest value of parthenogenetic development was recorded in DNB_4 (96.80%) followed by

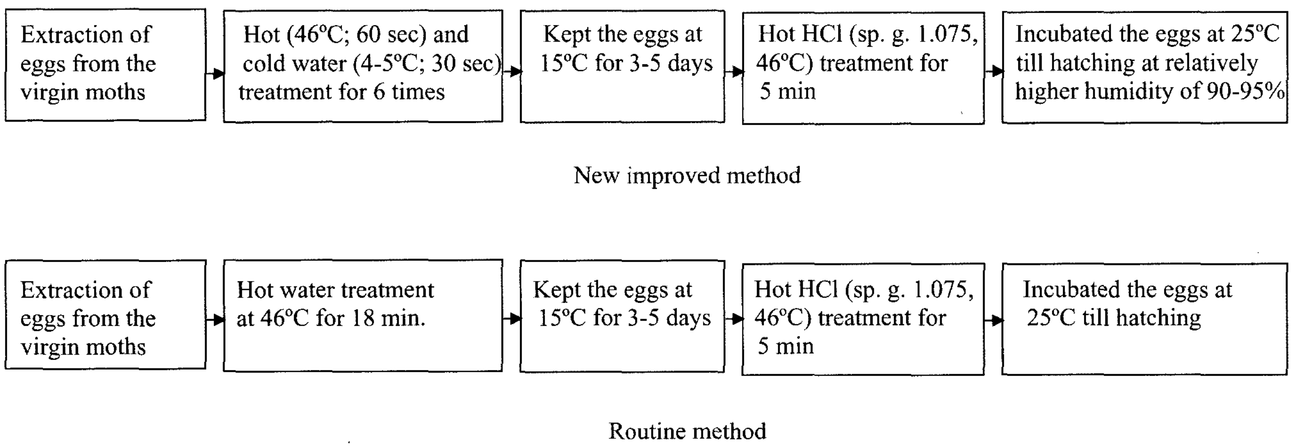

Fig. 1. A schematic representation of the new improved and routine method for parthenogenetic development in the silkworm, *B. mori* L.

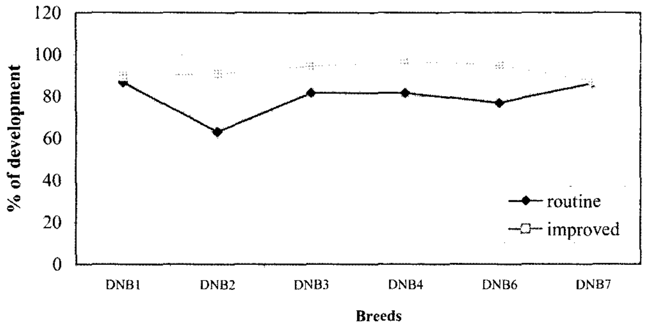

Fig. 2. Percentage of parthenogenetic development in routine and improved method in different breeds of the silkworm, *Bombyx mori* L.

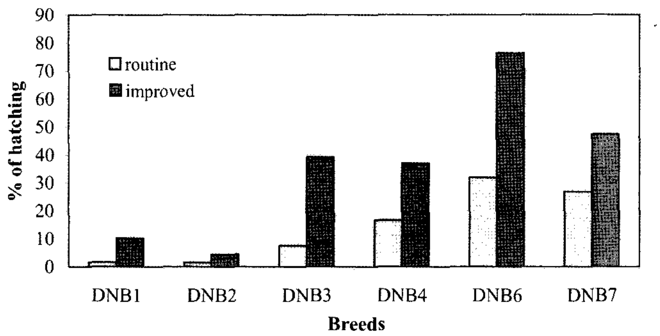

Fig. 3. Percentage of hatchability in routine and improved method in different breeds of the silkworm, *Bombyx mori* L.

DNB_6 (95.08%) and DNB_3 (94.54%). A higher trend towards parthenogenesis was observed in the new improved method where it was above 85% in all the breeds investigated. In the routine method, the percentage of parthenogenetic development ranged from 63.07% (DNB_2) to 86.82% (DNB_1).

Hatchability in these lines in the new and routine method was also studied. Data were computed and graphically represented in Fig. 3. A great deal of variation was observed among the different breeds tested. In the new method, the rate of hatchability was the highest in DNB_6 (76.36%) followed by DNB_7 (47.44%) and DNB_3 (39.26%). In the routine method, hatchability was relatively lower in the order of 31.95% (DNB_6), 26.82% (DNB_7) and 16.71% (DNB_4) respectively. The results of the present study clearly revealed that induction of parthenogenetic development and hatchability were more pronounced in the new method over the routine method.

Analysis of variance for combining ability computed for 12 economic characters namely, fecundity, hatching, pupation rate, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length, denier, reelability, raw silk and neatness showed a great deal of variations among the treatments, parents (lines), parents (testers), parents (lines vs. testers), parents vs. hybrids and hybrids (Table 1). A highly significant difference was observed in the treatments, parents, parents (lines) and hybrids for all the 12 characters under study. Whereas, highly significant difference for 7 characters *viz.*, pupation rate, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length and denier and significant difference for 2 characters *viz.*, hatching and raw silk were noticed in the testers. On the contrary, highly significant variations for 9 characters *viz.*, fecundity, hatching, pupaton rate, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, filament length, denier and raw silk percentage in lines vs. testers and significant and highly significant difference for almost all the characters except cocoon shell ratio in parents vs. hybrids was observed.

Percent contribution of lines, testers and lines × testers was presented in Table 2. Highest percentage of contribution towards fecundity (61.63%) followed by cocoon shell ratio (54.69%) and raw silk percentage (49.21%) was noticed in the lines. While it was highest for pupation rate (45.63%) followed by cocoon weight (36.57%) and reelability (36.47%) in the testers. Lines × testers revealed their excellence for denier (82.74%) followed by hatching (75.72%) and neatness (71.01%).

General combining ability (GCA) effects of lines and testers

Expression of general combining ability (GCA) effects of lines and testers is given in Table 3 and 4 respectively. From the Table 3, it is clear that DNB_1 exhibited significant GCA effects for 8 characters *viz.*, pupation rate, yield/10,000 larvae by weight, cocoon shell weight, cocoon shell ratio, filament length, denier, raw silk percentage and neatness followed by DNB_4 for 5 characters *viz.*, cocoon weight, cocoon shell weight, filament length, reelability and raw silk percentage. Among testers, CSR_2 expressed significant GCA effects for 7 characters followed by CSR_{17} for 4 characters (Table 4).

Specific combining ability (SCA) effects of hybrids

Specific combining ability effects for 24 hybrids have been presented in Table 5. Five hybrids *viz.*, $DNB_1 \times CSR_2$, $DNB_4 \times CSR_4$, $DNB_4 \times NB_4D_2$, $DNB_6 \times CSR_2$ and $DNB_7 \times CSR_2$ showed significant SCA for 5-6 characters whereas SCA estimated for four characters in 4 hybrids namely, $DNB_1 \times CSR_4$, $DNB_2 \times CSR_4$, $DNB_3 \times CSR_2$ and $DNB3 \times CSR_{17}$. On the contrary, no significant SCA effects were recorded in the hybrids of $DNB_1 \times NB_4D_2$, $DNB_2 \times CSR_2$, $DNB_4 \times CSR_2$ and $DNB_6 \times NB_4D_2$. Though, no significant SCA effects was found in the hybrids of $DNB_1 \times NB_4D_2$ and $DNB_6 \times NB_4D_2$, more interestingly,

Table 1. ANOVA for combining ability of 12 characteristics in some bivoltine × bivoltine hybrids of the silkworm, *Bombyx mori* L.

Source of variations	DF	Anova for Mean of Squares											
		Fecundity	Hatching	Pupation rate	Yield/ 10,000 larvae by wt.	Coccoon wt.	Cocoon shell wt.	Cocoon shell ratio	Filament length	Denier	Reela-bility	Raw silk	Neatness
Replicates	2	1529.25	15.85**	1.61	0.07	0.00	0.00	0.13	114.35	0.00	14.70*	0.31	0.48
Treatments	33	8270.02***	232.13***	72.66***	5.62***	0.04***	0.00***	1.39***	12706.00**	0.11***	44.10***	2.12***	7.58***
Parents	9	7094.46***	724.82***	53.82***	8.04***	0.06***	0.00***	1.93***	4878.11***	0.15***	19.89***	1.53***	4.95***
Parents (Lines)	5	6657.17***	1073.44***	34.91***	5.01***	0.07***	0.00***	2.80***	3955.16***	0.10***	30.89***	1.95***	8.00***
Parents (Testers)	3	2504.08	8.816*	54.41***	11.33***	0.02***	0.00***	0.98**	4448.97***	0.06***	7.28	0.57*	1.44
Parents (L vs. T)	1	23052.05***	1129.76***	146.52***	13.38***	0.11***	0.01***	0.42	10780.27***	0.64***	2.76	2.30***	0.20
Parents vs. hybrids	1	18262.61**	984.37***	268.51***	14.09***	0.24***	0.01***	0.10	103740.00***	0.03**	30.82**	1.14*	2.31*
Hybrids	23	8295.57***	6.63***	71.51***	4.31***	0.03***	0.00***	1.23***	11811.08***	0.11***	54.15***	2.39***	8.84***
Error	66	1587.16	2.38	6.68	0.27	0.00	0.00	0.23	192.53	0.00	4.02	0.17	0.53
Total	101	3769.53	77.71	28.13	2.01	0.02	0.00	0.60	4279.54	0.04	17.32	0.81	2.83

*, ** and *** denote significant difference at 5%, 1% and 0.1% level respectively.

Table 2. Percent contribution of lines, testers and line × testers of the bivoltine silkworm, *Bombyx mori* L.

Source	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Coccoon wt.	Cocoon shell wt.	Cocoon shell ratio	Filament length	Denier	Reelability	Raw silk	Neatness
Line	61.63	11.14	16.26	24.23	17.20	34.10	54.69	26.42	10.81	11.09	49.21	10.12
Tester	7.56	13.14	45.63	35.53	36.57	32.79	9.67	14.97	6.44	36.47	13.88	18.87
Line × testers	30.80	75.72	38.12	40.24	46.23	33.11	35.64	58.62	82.74	52.45	36.91	71.01

Table 3. General combining effects in lines of the bivoltine silkworm, *Bombyx mori* L. utilized in the present study

Parents (Lines)	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Coccoon wt.	Cocoon shell wt.	Cocoon shell ratio	Filament length	Denier	Reelability	Raw silk	Neatness
DNB_1	-2.50	-0.04	2.60**	1.19***	0.03	0.024***	0.94***	44.54***	-0.082**	-2.27	1.14***	0.68**
DNB_2	7.50	-0.11	0.91	-0.10	0.01	-0.002	-0.22	3.63	-0.024	0.54	-0.43	-0.49
DNB_3	-49.50	-0.63	-2.85	-0.47	-0.02	-0.013	-0.54	-60.04	-0.002	-0.62	-0.76	0.26
DNB_4	-37.75	0.60	-0.12	-0.01	0.04*	0.009**	0.10	18.21***	0.117	2.35**	0.36**	0.35
DNB_6	76.33***	0.66	1.51*	-0.01	-0.08	-0.017	-0.06	-6.79	-0.025	-0.25	-0.05	0.10
DNB_7	5.92	-0.48	-2.05	-0.59	0.02	-0.001	-0.22	0.46	0.016	0.25	-0.27	-0.90
CD@5%	24.61	0.88	1.49	0.28	0.04	0.006	0.26	6.55	0.033	1.24	0.24	0.39
CD@1%	32.85	1.17	1.99	0.37	0.05	0.009	0.34	8.74	0.044	1.65	0.32	0.53
SE	12.22	0.44	0.74	0.14	0.02	0.003	0.13	3.25	0.016	0.61	0.12	0.20

*, ** and *** denote significant difference at 5%, 1% and 0.1% level respectively.

Table 4. General combining effects in testers of the bivoltine silkworm, *Bombyx mori* L. utilized in the present study

Parents (Testers)	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Coccoon wt.	Cocoon shell wt.	Cocoon shell ratio	Filament length	Denier	Reelability	Raw silk	Neatness
CSR_2	-20.44	0.11	4.37***	1.16***	0.06**	0.015***	0.20	31.54***	0.052	3.67***	0.35**	-0.01
CSR_4	-4.17	0.82*	-1.04	-0.54	-0.08	-0.014	0.08	-14.13	0.029	0.79	0.27**	1.15***
CSR_{17}	6.67	-0.44	-4.49	-0.55	0.06**	0.012**	0.05	12.65***	-0.071***	-1.45	-0.16	-0.29
NB_4D_2	17.94	-0.49	1.16	-0.07	-0.04	-0.013	-0.32	-30.07	-0.009	-3.01	-0.46	-0.85
CD@5%	20.09	0.72	1.22	0.23	0.03	0.005	0.21	5.34	0.027	1.01	0.19	0.32
CD@1%	26.82	0.96	1.63	0.30	0.04	0.007	0.28	7.13	0.036	1.35	0.26	0.43
SE	9.98	0.36	0.61	0.11	0.01	0.003	0.10	2.66	0.013	0.50	0.10	0.16

*, ** and *** denote significant difference at 5%, 1% and 0.1% level respectively.

Table 5. Specific combining effects in F_1 hybrids of the bivoltine silkworm, *Bombyx mori* L. utilized in the present study

Hybrids	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Coccoon wt.	Cocoon shell wt.	Cocoon shell ratio	Filament length	Denier	Reelability	Raw silk	Neatness
$DNB_1 \times CSR_2$	-0.56	-0.12	-5.03	-0.89	-0.08	-0.005	0.65*	25.63**	-0.38***	-1.63	0.67**	1.60**
$DNB_1 \times CSR_4$	-15.17	0.02	2.02	0.76**	0.06	0.015*	0.21	23.96**	0.22	1.11	0.69**	0.76
$DNB_1 \times CSR_{17}$	5.33	1.58	2.80	0.01	-0.02	-0.020	-0.86	-3.82	-0.13**	0.51	-1.40	-0.79
$DNB_1 \times NB_4D_2$	10.39	-1.48	0.22	0.12	0.05	0.010	0.00	-45.76	0.29	0.01	0.04	-1.57
$DNB_2 \times CSR_2$	37.11	-1.79	1.23	0.03	0.02	-0.001	-0.29	-74.13	0.17	-7.32	-0.31	-1.57
$DNB_2 \times CSR_4$	35.17	0.41	2.24	0.88**	0.05	0.006	-0.26	89.21***	-0.16**	0.46	-0.34	0.93*
$DNB_2 \times CSR_{17}$	-7.00	1.39	-1.38	-0.31	-0.01	-0.005	-0.16	0.24	0.17	3.66**	0.15	2.04**
$DNB_2 \times NB_4D_2$	-65.28	0.00	-2.09	-0.59	0.06	0.001	0.71**	-14.85	-0.18***	3.20*	0.49*	-1.40
$DNB_3 \times CSR_2$	17.44	-0.05	1.39	0.91**	0.06	0.005	-0.35	19.88**	0.26	2.79*	-0.02	1.01*
$DNB_3 \times CSR_4$	16.83	-0.66	-0.39	-1.08	-0.16	-0.023	0.43	-47.13	-0.08*	1.29	0.17	-2.15
$DNB_3 \times CSR_{17}$	-12.00	0.81	0.92	0.82**	0.12**	0.029**	0.26	36.43***	-0.04	-2.04	0.25	-1.38
$DNB_3 \times NB_4D_2$	-22.28	-0.10	-1.92	-0.64	-0.03	-0.010	-0.34	-9.18	-0.14**	-2.04	-0.40	2.51***
$DNB_4 \times CSR_2$	-57.31	-0.47	-5.34	-1.15	-0.06	-0.013	-0.134	-55.38	-0.01	-4.42	-1.02	-2.40
$DNB_4 \times CSR_4$	-25.58	0.66	5.68**	1.46**	0.07*	0.020**	0.30	45.29***	-0.03	-5.99	0.66**	-0.90
$DNB_4 \times CSR_{17}$	30.92	-0.28	-0.88	-0.19	-0.03	-0.005	0.08	-39.82	0.14	2.52	0.60*	2.21***
$DNB_4 \times NB_4D_2$	51.97*	0.09	0.54	-0.12	0.01	-0.002	-0.25	49.90***	-0.09**	3.89**	-0.24	1.10**
$DNB_6 \times CSR_2$	-25.39	-0.21	2.63	0.56*	0.10**	0.025**	0.21	62.96***	-0.05	2.94*	0.08	0.85
$DNB_6 \times CSR_4$	-4.33	-0.45	-5.56	-0.56	0.02	-0.008	-0.66	-28.04	0.05	3.02*	-0.66	0.35
$DNB_6 \times CSR_{17}$	18.50	0.62	0.29	-0.42	-0.09	-0.013	0.29	24.51**	-0.24***	-4.01	0.36	-1.54
$DNB_6 \times NB_4D_2$	11.22	0.04	2.64	0.41	-0.03	-0.003	0.16	-59.43	0.24	-1.95	0.22	0.35
$DNB_7 \times CSR_2$	28.69	2.65**	5.13**	0.55	-0.05	-0.011	-0.09	21.04**	-0.00	3.64**	0.60*	0.51
$DNB_7 \times CSR_4$	-6.92	0.02	-3.99	-1.46	-0.05	-0.009	-0.01	-83.29	0.01	0.12	-0.53	1.01*
$DNB_7 \times CSR_{17}$	-35.75	-4.12	-4.49	0.09	0.04	0.015*	0.38	-17.07	0.11	-0.65	0.03	-0.54
$DNB_7 \times NB_4D_2$	13.97	1.45	1.16	0.82**	0.05	0.005	-0.29	79.32***	-0.11**	-3.11	-0.11	-0.99
CD@5%	49.21	1.75	2.99	0.56	0.07	0.013	0.52	13.09	0.07	2.47	0.47	0.79
CD@1%	65.69	2.34	3.99	0.74	0.10	0.017	0.69	17.48	0.09	3.30	0.63	1.05
SE	24.45	0.87	1.48	0.28	0.04	0.006	0.26	6.50	0.03	1.23	0.24	0.39

*, ** and *** denote significant difference at 5%, 1% and 0.1% level respectively.

Table 6. Heterosis over mid- and better parent values in F_1 hybrids of the bivoltine silkworm, *Bombyx mori* L. utilized in the present study

Hybrids	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Coccoon wt.	Cocoon shell wt.	Cocoon shell ratio	Filament length	Denier	Reelability	Raw silk	Neatness
$DNB_1 \times CSR_2$												
MPV	-2.78	-0.70	0.18	2.12	3.46	9.41**	5.73**	16.54**	-19.93**	-1.86	9.71**	0.54
BPV	-3.16	-1.34	-0.61	-4.31	2.84	4.37	1.51	12.26**	-22.14**	-2.77	8.92**	0.00
$DNB_1 \times CSR_4$												
MPV	0.53	-0.24	7.32**	18.63**	8.99*	13.96**	5.00*	12.30**	-2.07	-1.97	12.61**	1.28*
BPV	-2.84	-0.47	2.77	9.16**	3.22	2.05	-1.07	6.82**	-6.00**	-2.94	8.58**	0.36
$DNB_1 \times CSR_{17}$												
MPV	0.13	-1.07	-0.73	2.78	9.90**	5.50*	-3.74	10.53**	-12.06**	-7.07	-6.48	-1.82
BPV	-2.79	-1.96	-1.30	1.34	6.14	-0.71	-6.27	6.70**	-13.29**	-7.68	-7.47	-2.89
$DNB_1 \times NB_4D_2$												
MPV	4.66	-2.23	4.59	8.58**	12.25**	11.33**	-0.61	5.70**	0.97	-7.55	2.73	-2.93
BPV	2.99	-3.38	3.21	8.04*	5.12	0.89	-3.97	-3.03	-1.44	-9.02	-0.21	-4.33
$DNB_2 \times CSR_2$												
MPV	-5.16	-1.18	8.85**	7.12*	9.91**	8.18**	-1.65	4.37**	7.82	-5.78	-1.08	-3.12
BPV	-14.76	-1.88	4.31	-6.39	8.90*	7.81*	-2.83	3.72*	-2.09	-6.98	-5.90	-3.65
$DNB_2 \times CSR_4$												
MPV	-0.03	1.46	9.57**	19.81**	9.11*	8.69**	-0.16	20.63**	-6.57**	0.42	1.26	1.29*
BPV	-12.53	0.32	8.62*	18.75**	4.85	1.37	-3.25	19.80**	16.18	-0.94	-0.87	1.10
$DNB_2 \times CSR_{17}$												
MPV	-10.87	0.01	-3.83	-0.39	10.86**	7.36*	-3.15	10.98**	7.94	-0.06	-1.01	1.11
BPV	-17.40	-2.22	-7.65	-8.78	8.68*	5.47	-3.40	10.04**	1.92	-0.37	-5.53	1.11
$DNB_2 \times NB_4D_2$												
MPV	-17.71	0.63	3.75	3.10	5.80	6.00*	0.13	9.52**	-6.82**	-0.34	1.24	-2.96
BPV	-24.70	0.46	1.50	-3.91	0.51	0.10	-0.42	4.73**	-15.09**	-2.26	-1.62	-3.32
$DNB_3 \times CSR_2$												
MPV	-11.89	0.45	4.64	10.41**	14.29**	10.96**	-2.98	7.55**	10.88	8.44**	-2.53	0.37
BPV	-15.41	-0.59	0.43	-3.53	9.44*	6.98*	-3.62	7.48**	1.54	6.75*	-6.23	0.00
$DNB_3 \times CSR_4$												
MPV	-6.28	0.12	1.82	2.08	-1.95	0.33	2.34	-5.20	-3.55*	2.91	1.30	-1.47
BPV	-12.60	-1.33	0.78	1.21	-2.49	-2.75	-0.29	-6.37	-12.75**	1.39	0.34	-1.47
$DNB_3 \times CSR_{17}$												
MPV	-15.17	-0.82	-5.63	4.84	21.50**	18.94**	-2.13	7.02**	-0.06	-5.88	-3.75	-2.03
BPV	-15.83	-3.34	-9.24	-4.01	19.71**	16.30**	-2.92	6.71**	-4.80*	-8.78	-7.09	-2.21
$DNB_3 \times NB_4D_2$												
MPV	-13.82	0.30	-0.54	0.14	10.23**	3.45	-6.22	1.31	-5.56**	-5.69	-8.36	2.03*
BPV	-15.65	-0.20	-2.55	-6.69	8.32*	1.59	-6.22	-3.64	-13.21**	-6.54	-9.91	1.47*

Table 6. Continued

Hybrids	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Coccoon wt.	Cocoon shell wt.	Cocoon shell ratio	Filament length	Denier	Reelability	Raw silk	Neatness
DNB$_4$ × CSR$_2$												
MPV	-25.70	1.66	1.71	2.40	11.22**	11.85**	0.44	5.24**	4.28	8.79**	-4.79	-1.49
BPV	-30.40	0.25	-3.88	-12.48	5.93	8.16*	-1.08	2.76*	-3.41	6.45**	-5.41	-3.65
DNB$_4$ × CSR$_4$												
MPV	-14.61	3.17*	14.08**	28.30**	16.79**	21.46**	4.05*	13.34**	1.00	-2.03	8.77**	1.87*
BPV	-22.23	1.33	13.40**	26.10**	16.79**	17.37**	0.53	9.26**	-7.61**	-4.05	6.29*	0.00
DNB$_4$ × CSR$_{17}$												
MPV	-7.77	-0.34	-3.09	3.48	16.03**	15.07**	-0.70	4.61**	9.83	4.26	2.83	3.94**
BPV	-10.78	-3.20	-8.23	-7.44	13.69	12.85**	-0.76	2.36	5.88	0.45	2.52	2.21**
DNB$_4$ × NB$_4$D$_2$												
MPV	-0.77	2.18	7.11*	9.62**	16.90**	13.12**	-3.37	16.13**	-0.54	6.46*	-2.84	2.45**
BPV	-5.28	1.30	3.30	-0.26	15.52	10.74**	-4.21	7.92**	-7.55**	4.87	-4.34	1.12
DNB$_6$ × CSR$_2$												
MPV	-1.39	11.55**	13.04**	3.28	1.47	5.33*	3.70	17.34**	-7.22**	5.01*	4.02	0.00
BPV	-9.36	0.59	6.47*	-2.86	-4.56	-0.35	3.00	15.33**	-9.47**	2.70	-0.99	-0.36
DNB$_6$ × CSR$_4$												
MPV	8.19	11.57**	2.90	0.51	-6.09	-5.82	0.58	2.00	-6.07**	1.63	1.61	1.10
BPV	-3.27	0.23	1.91	-7.85	-15.61	-16.32	-0.71	-1.04	-9.54**	-0.69	-0.46	1.10
DNB$_6$ × CSR$_{17}$												
MPV	7.78	9.93**	0.46	-7.94	-6.65	-4.79	1.79	10.11**	-14.25**	-11.32	2.92	-2.39
BPV	2.23	-2.23	-5.19	-8.86	-14.55	-11.14	-0.35	8.46**	-15.74**	-11.89	-1.71	-2.57
DNB$_6$ × NB$_4$D$_2$												
MPV	9.88	11.80**	11.86**	2.28	-5.82	-5.46	0.08	-0.23	0.85	-8.69	1.86	-0.55
BPV	2.89	1.30	7.50*	1.37	-16.26	-15.00	-1.25	-6.71	-1.22	-11.29	-0.95	-1.10
DNB$_7$ × CSR$_2$												
MPV	2.28	36.62**	11.06**	2.59	1.54	5.64*	3.87	17.68**	-2.70	5.97*	7.10**	-1.47
BPV	-0.25	2.41	5.32	-6.24	-1.79	5.38	0.65	15.13**	-6.61**	3.15	1.01	-1.82
DNB$_7$ × CSR$_4$												
MPV	1.56	33.17**	-0.24	-6.74	-1.38	4.40	5.81*	0.00	-4.52*	-1.83	1.97	0.74
BPV	-3.83	-0.46	-0.48	-11.99	-9.00	-2.54	4.53	-0.89	-9.54**	-4.51	-1.05	0.74
DNB$_7$ × CSR$_{17}$												
MPV	-7.96	23.72**	-6.61	-5.62	9.34**	14.03**	3.96*	10.20**	1.14	-7.06	0.07	-2.39
BPV	-8.74	-8.21	-11.26	-9.36	2.83	12.13**	-0.68	7.57**	1.08	-8.10	-5.31	-2.57
DNB$_7$ × NB$_4$D$_2$												
MPV	4.69	35.06**	4.57	4.35	7.95*	7.76*	-0.60	23.50**	-8.18**	-9.97	-0.98	-3.14
BPV	4.14	1.60	1.20	2.09	-1.47	1.86	-4.30	19.92**	-11.54**	-12.93	-4.62	-3.68
CSR$_2$ × CSR$_4$												
MPV	10.44	0.12	2.34	8.19**	15.49**	19.81**	3.80*	17.15**	-3.10*	-4.68	2.72	1.47*
BPV	7.14	-0.30	-2.73	-6.16	9.99**	12.09**	1.79	15.63**	-4.39*	-4.76	-0.26	1.09

* and ** denote significant difference at 5% and 1% level respectively.

positive value for 7-8 characters was observed in those hybrids.

Hybrid vigour studies

Hybrid vigour over mid and better parent value in different F_1 hybrids of the bivoltine breeds has been computed in Table 6. The hybrid, $DNB_4 \times CSR_4$ was identified as promising and expressed significant hybrid vigour over better parent for 7 quantitative characters *viz.*, pupation rate, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, filament length, denier and raw silk percentage. Four hybrids (*viz.*, $DNB_1 \times CSR_4$, $DNB_3 \times CSR_2$, $DNB_3 \times CSR_{17}$ and $CSR_2 \times CSR_4$) recorded significant hybrid vigour over better parent for four characters while significant hybrid vigour over BPV for 3 characters was observed in other 7 hybrids *viz.*, $DNB_1 \times CSR_2$, $DNB_2 \times CSR_2$, $DNB_2 \times CSR_4$, $DNB_3 \times NB_4D_2$, $DNB_4 \times CSR_2$, $DNB_4 \times NB_4D_2$ and $DNB_6 \times CSR_2$. Majority of the hybrids expressed significant hybrid vigour for pupation rate, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, filament length and denier. Significant hybrid vigour for hatching was noticed in the hybrid progeny when DNB_6 and DNB_7 were kept as female component.

Discussion

In the present study, practical advantages of artificial parthenogenesis in the exploitation of heterosis have been assessed based on combining ability analysis and hybrid vigour studies. Practical advantages of artificial parthenogenesis in hybrids comprising parthenoclone as a component showed more viability, hybrid vigour, combining ability and phenotypically uniform population (Strunnikov *et al.*, 1982; Strunnikov, 1986; Takei *et al.*, 1990; Ravindra Singh *et al.*, 1994). Mechanism of hybrid vigour through artificial parthenogenesis has also been analyzed (Ohkuma, 1971). Perusal of data revealed that great deal of variations exists among the hybrids studied. Analysis of variance for combining ability computed for lines exhibited highly significant differences for all the characters, establishing the fact of existence of genotypic differences in their expression and may be attributed due to variations in genetic make-up of the silkworm breeds utilized. Significant and highly significant differences in parents vs. hybrids may be assumed due to the high degree of divergence exist among the lines. Percent contribution of lines was found highest for fecundity (61.63%) whereas it was highest for pupation rate (45.63%) in the testers. Contrarily, lines × testers showed their excellence for denier (82.74%) followed by hatching (75.72%) and neatness (71.01%).

Among the lines, DNB_1 was found as best general combiner and exhibited significant GCA effects for 8 characters followed by DNB_4 for 5 characters indicating major role of additive gene action for expression of these characters. Interaction of additive gene action and its influence has been reported on cocoon yield (Subba Rao and Sahai, 1989; Ravindra Singh *et al.*, 2001), cocoon weight, cocoon shell weight and cocoon shell ratio (Satenahalli *et al.*, 1989; Rajalakshmi *et al.*, 1997). Highly significant GCA effect for fecundity (76.33) was noticed in the line, DNB_6. Predominant role of additive gene action has been reported for fecundity (Jolly, 1983). Among testers, CSR_2 and CSR_{17} were found as good general combiners and recorded significant GCA effect for 7 characters and 4 characters respectively. Major role of additive and non-additive gene action for the expression of pupation rate and filament length has been reported (Kumar *et al.*, 1994).

Superiority of F_1 hybrids is judged through specific combining ability analysis and hybrid vigour studies. Estimated values of specific combining ability in F_1 hybrids are presented in Table 5. Five hybrids *viz.*, $DNB_1 \times CSR_2$, $DNB_4 \times CSR_4$, $DNB_4 \times NB_4D_2$, $DNB_6 \times CSR_2$ and $DNB_7 \times CSR_2$ showed significant SCA for 5-6 characters Whereas, significant SCA for four characters was found in the 4 hybrids namely, $DNB_1 \times CSR_4$, $DNB_2 \times CSR_4$, $DNB_3 \times CSR_2$ and $DNB_3 \times CSR_{17}$. Majority of the hybrids revealed significant SCA effects for yield/10,000 larvae by weight, cocoon shell weight, filament length, denier, reelability, raw silk percentage and neatness. From the above observation it may be assumed that in addition to the effects of non-additive gene action, epistatic effects of additive × additive and complimentary genes have also played major role in the manifestation of these characters as the parents involved are high × high, high × medium and high × low GCA contributors. Similar results were reported in the bittergourd (Ram *et al.*, 1999) and polyvoltine × bivoltine silkworm (Rao *et al.*, 2002). Predominant role of non-additive gene action for expression of cocoon weight, cocoon shell weight and cocoon shell ratio percentage was noticed by several workers (Pershad *et al.*, 1986; Bhargava *et al.*, 1995; Ravindra Singh *et al.*, 2000; Datta *et al.*, 2001). Recently, Eid *et al.* (2005) observed the effect of non-additive gene action in the manifestation of larval and cocoon characters through combining ability estimates.

Analysis of hybrid vigour in 25 bivoltine F_1 hybrids demonstrated superiority of the hybrid, $DNB_4 \times CSR_4$ exhibiting significant hybrid vigour over better parent value for 7 quantitative characters out of 12 characters studied. Eleven hybrids expressed significant hybrid vigour over BPV for 3-4 quantitative characters. Fonseca and Patterson (1968) emphasized that in the utilization of heterosis in commercial crops, hybrid vigour over better parent considered significance. Majority of the hybrids exhibited significant hybrid vigour for survival rate, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight, filament length and denier. Similar results in the expression of significant hybrid vigour was observed for cocoon yield, survival rate, cocoon weight, filament length and denier in bivoltine hybrids (Subba Rao and Sahai, 1989) and cocoon yield, cocoon weight, cocoon shell weight in polyvoltine × bivoltine hybrids (Rao *et al.*, 2002). Recently, heterosis over mid and better parent for cocoon yield, cocoon weight, and cocoon shell weight was reported through line × tester analysis in the evaluation and identification of prospective polyvoltine × bivoltine hybrids of the silkworm (Ravindra Singh *et al.*, 2005).

Combining ability analysis and hybrid vigour studies in the newly developed bivoltine breeds/hybrids for 12 quantitative characters demonstrated that the genotype, DNB_1 adjudicated as best line followed by DNB_4. Among the testers, CSR_2 and CSR_{17} were found as good general combiners. Four heterotic hybrids *viz.*, $DNB_4 \times CSR_4$, $DNB_1 \times CSR_4$, $DNB_3 \times CSR_2$ and $DNB_3 \times CSR_{17}$ were identified as promising for the desirable characters. These hybrids will be further evaluated on large scale and one promising hybrid will be short listed and recommended for commercial exploitation.

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