

Boil-off Loss Ratio of Cocoon Shell as a Selection Criterion in the Newly Developed Bivoltine Silkworm (*Bombyx mori* L.) Hybrids

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Abstract

Boil-off loss ratio is one of the most important economic traits to be considered during the course of silkworm breeding. The boil-off loss ratio varies among the breeds and seasons. The present study was undertaken with 5 oval and 5 peanut breeds and twenty five hybrids involving these parents to select the promising hybrids with desired boil-off loss ratio. Accordingly, studies were undertaken to estimate the heterosis and heterobeltiosis. It was observed that majority of the hybrids revealed negative heterosis for boil-off loss ratio which is desirable. Based on the heterosis and heterobeltiosis, among all the hybrids, the hybrid APBRO5 x APBRD5 exhibited highest negative heterosis for boil-off loss ratio and also exhibited highest EI value (64.5) for the quantitative and qualitative traits. Based on the hybrids performance, boil-off loss ratio, heterosis and heterobeltiosis and evaluation index, the hybrids viz. APBRO5 x APBRD5, APBRO1 x APBRD1 and APBRO3 x APBRD4 were identified for commercial exploitation.

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Introduction

To face the global competitiveness in silk production there is a need to improve the quality of the raw silk and as such the sericulture industry requires good quality of silk to produce internationally gradable silk fabric to meet the requirement of the consumers across the world resulting in increased foreign exchange. To meet the specific requirement of the silk industry, the silkworm breeders have developed various hybrids such as productive breeds, robust breeds,

breeds with special traits and sex limited races etc. Keeping this in view, most of the silkworm breeders have been concentrating on boil-off loss ratio to produce good quality silk as the boil-off loss ratio is one of the most important qualitative trait in silkworm breeding. The silkworm *Bombyx mori* L. spins a shell by extruding silk bave at the end of its larval period. The silk bave is composed of two silk proteins namely fibroin and sericin and also contains a small quantity of fatty, waxy, colouring and mineral matters. The fibroin protein occupies the middle portion of the

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silk bave representing 70-80 % of its weight and surrounded by three layers of sericin which is representing 20-30 % of the weight. The fatty, waxy, colouring and mineral matters forms a very small part of the silk bave which is not more than 2-3 %.

The main silk substance fibroin is insoluble in alkaline hot water, whereas the sericin (silk gum) is easily soluble in boiling alkaline soap solution (Sadov *et al.*, 1978). Degumming is the process of removal of sericin in boiling soap solution. The cocoon shell has more boil-off loss percentage when compared to the raw silk. The percentage of boil-off loss ratio has paramount importance in reeling and weaving activities (Kannan, 1986).

The loss of sericin varies in different races and also there is difference in the effect of sericin loss from raw silk on weaving and other post weaving process due to degumming (Sinha *et al.*, 1992). The boil-off loss ratio for bivoltine is found to be 24 % and it is higher in polyvoltines (Sidhu and Sonwalkar, 1969). The boil-off loss ratio varies according to seasons is influenced by the environment (Sonwalkar, 1969). It is essential to study the boil-off loss ratio with reference to cocoon shell, as it is the basic raw material for the raw silk. The present study was aimed in estimating the boil-off loss ratio in newly developed hybrids as well as their parents for selecting the promising bivoltine hybrids for the benefit of the silk industry.

Materials and Methods

Five oval breeds *viz.* APBRO1, APBR02, APBR03, APBRO4 and APBRO5 (APBRO means Andhra Pradesh Boil – off loss Ratio Oval breeds) and five dumbbell breeds *viz.* APBRD1, APBRD2, APBRD3, APBRD4 and APBRD5 (APBRD means Andhra Pradesh Boil – off loss Ratio Dumbbell breeds) were selected for the study. All these parental breeds (5 oval and 5 dumb-bell) and twenty five hybrids were reared by the standard rearing techniques (Basavaraja and Dandin, 2002). After cocoon formation the sex-

separation and assessment, 60 cocoon shells (30 each of female and male) were selected from each breed/ hybrid separately. Three replications were maintained each having 10 males and 10 females for conducting the experiment. Degumming was carried out by boiling the cocoon shells in soap solution using a standard procedure (Basavaraja *et al.*, 2000). Two step boiling method as explained below was employed for the degumming of the cocoon shells and used neutral liquid soap for boiling. A quantity of 7g soap was dissolved in 1 liter of water for degumming and the material: liquor ratio was maintained as 1:40.

First degumming

The liquor solution of the soap and soda was boiled in a copper vessel. When the boiling media solution reached the boiling point ($>90^{\circ}\text{C}$), the cocoon shell sample bags were immersed completely in boiling media kept tightly closed for 40 minutes. At an interval of 10 minutes, the sample bags were turned up and down for uniform and effective degumming.

Second degumming

In another container, the boiling media of the required concentration was prepared and kept ready at boiling point ($>90^{\circ}\text{C}$), to transfer the sample material after the first boiling. Immediately after 40 minutes of the first boiling, the sample bags were removed and squeezed to remove the excess liquor solution and transferred to the second bath. The degumming was carried out for another 40 minutes similar to the first degumming as explained above.

Rinsing

Immediately after the second boiling, the sample bags with the degummed shells were rinsed for a minute in an already prepared boiling alkaline solution of 0.1 % sodium carbonate.

Washing

After rinsing in 0.1 % sodium carbonate solution, the sample bags with cocoon shells were washed thoroughly (for 15 minutes) in running water using a washing machine. Then, they were dried in the washing machine itself for 5 minutes to remove the excess moisture.

Drying and weighing

The degummed cocoon shells were transferred to perforated paper envelopes and kept for drying in the oven at 105°C for 5 hours. The dried sample packets were transferred to the desiccator for half an hour for absorption of excess moisture before recording the dry weight of the degummed silk.

The boil-off loss ratio percentage was calculated by using the formula:

$$\text{Boil-off loss ratio} = \frac{\text{Initial dry weight} - \text{Final dry weight after degumming}}{\text{Initial dry weight}} \times 100$$

The heterosis and heterobeltiosis were calculated by using the following formulae:

$$\text{Heterosis percentage over mid parental value (MPV)} =$$

$$\frac{F1 - MPV}{MPV} \times 100$$

Heterobeltiosis (over-dominance) over better parental value

$$BPV = \frac{F1 - BPV}{BPV} \times 100$$

Multiple Trait Evaluation Index (Mano *et al.*, 1992) was calculated by using the following formula:

$$\text{Evaluation index} = \frac{A - B}{C} \times 10 + 50$$

Where,

A = Value obtained for a particular trait of a particular hybrid combination

B = Mean value of the particular trait of all the

Table 1. Mean performance of ten parental breeds

Breeds	Boil -off loss Ratio (%)
Ovals	
APBRO1	24.02
APBRO2	25.54
APBRO3	25.17
APBRO4	25.07
APBRO5	25.17
APS5	24.25
CD at 5 %	0.487
Dumbbells	
APBRD1	25.21
APBRD2	24.87
APBRD3	24.82
APBRD4	24.89
APBRD5	25.22
APS4	24.25
CD at 5 %	0.402

hybrid combinations

C = Standard deviation

10 = Standard unit and

50 = Fixed value

Results and Discussion

The boil-off loss ratio for the oval lines ranges from 24.02 % to 25.54 %. The variation in boil-off loss ratio among oval lines was less. Among oval lines the low boil-off loss ratio was recorded in APBRO1 (24.02 %) and maximum in APBRO2 (25.54 %). The average boil-off loss ratio of oval lines was 24.99% (Table 1). However, the entire oval breeds shown

Table 2. Mean Boil-off loss ratio of twenty five hybrids

Sl. No.	Hybrid	Boil-off loss ratio (%)
1	APBRO1 × APBRD1	23.34
2	APBRO1 × APBRD2	24.56
3	APBRO1 × APBRD3	25.43
4	APBRO1 × APBRD4	25.10
5	APBRO1 × APBRD5	25.73
6	APBRO2 × APBRD1	25.39
7	APBRO2 × APBRD2	25.75
8	APBRO2 × APBRD3	25.39
9	APBRO2 × APBRD4	25.29
10	APBRO2 × APBRD5	25.36
11	APBRO3 × APBRD1	25.03
12	APBRO3 × APBRD2	24.64
13	APBRO3 × APBRD3	24.37
14	APBRO3 × APBRD4	23.81
15	APBRO3 × APBRD5	24.16
16	APBRO4 × APBRD1	24.54
17	APBRO4 × APBRD2	24.63
18	APBRO4 × APBRD3	24.84
19	APBRO4 × APBRD4	24.55
20	APBRO4 × APBRD5	24.27
21	APBRO5 × APBRD1	24.02
22	APBRO5 × APBRD2	24.74
23	APBRO5 × APBRD3	24.61
24	APBRO5 × APBRD4	24.28
25	APBRO5 × APBRD5	24.43
C	APS5 × APS4	24.32
	CD at 5%	0.581

slight variation among them.

Among dumbbell lines the low boil-off loss ratio was recorded in APBRD3 (24.82%) followed by and maximum boil-off loss ratio was recorded in APBRD5 (25.22%). The average boil-off loss ratio of dumbbell lines was 25.00%.

In case of hybrids the minimum boil-off loss ratio was recorded in APBRO1 × APBRD1 (23.34%) and maximum was recorded in APBRO2 × APBRD2 (25.75%) as against 24.32% in the control hybrid APS5 × APS4 (Table 2). Among the hybrid combinations, with regard to heterosis over MPV maximum negative heterosis was recorded in APBRO1 × APBRD1 (-5.18) followed by APBRO3 × APBRD4 (-4.89), APBRO5 × APBRD1 (-4.66) and APBRO3 × APBRD5 (-4.12) where as minimum was in APBRO2 × APBRD5 (-0.08). With regard to heterosis over BPV, maximum negative heterosis was recorded in APBRO5 × APBRD1 (-4.57) followed by APBRO3 × APBRD4 (-4.35), APBRO3 × APBRD5 (-4.03), APBRO4 × APBRD5 (-3.18) and APBRO5 × APBRD5 (-2.93) where as minimum was in APBRO5 × APBRD2 (-0.52). However, eight hybrid combinations have recorded positive boil-off loss ratio over MPV and ten hybrid combinations over BPV which is not desirable for the industry (Table 3). All the hybrid combinations that are involved with the female component of APBRO3, APBRO5 and APBRO4 are showing negative heterosis and heterobeltiosis for the boil-off loss ratio. The performance of these lines regarding boil-off loss ratio is more as pure lines and as a hybrid it is less when compared to the other hybrid combinations which clearly indicates that, these are best combiners regarding the negative heterosis towards this trait. The superiority of the hybrids over parental strains is undoubtedly due to variable magnitude of heterosis for the quantitative and qualitative characters in silkworm and the results of present study are corroborating the findings of Osawa and Harada (1944), Katsumata (1948) and Yokoyama (1962). It has been pointed out by Toyama (1906) that the F1 hybrids in silkworm, *Bombyx mori* in several aspects are superior to their pure line parents and

Table 3. Heterosis and Over-dominance for Boil-off loss ratio in hybrids

Sl. No.	Hybrid	Heterosis (%) (over MPV)	Over-dominance (%) (over BPV)
1	APBRO1 × APBRD1	-5.18	-2.817
2	APBRO1 × APBRD2	0.46	2.248
3	APBRO1 × APBRD3	4.14	5.871
4	APBRO1 × APBRD4	2.63	4.497
5	APBRO1 × APBRD5	4.49	7.105
6	APBRO2 × APBRD1	0.05	0.701
7	APBRO2 × APBRD2	2.18	3.552
8	APBRO2 × APBRD3	0.85	2.297
9	APBRO2 × APBRD4	0.30	1.607
10	APBRO2 × APBRD5	-0.08	0.555
11	APBRO3 × APBRD1	-0.66	-0.583
12	APBRO3 × APBRD2	-1.53	-0.925
13	APBRO3 × APBRD3	-2.49	-1.800
14	APBRO3 × APBRD4	-4.89	-4.352
15	APBRO3 × APBRD5	-4.12	-4.025
16	APBRO4 × APBRD1	-2.40	-2.114
17	APBRO4 × APBRD2	-1.36	-0.952
18	APBRO4 × APBRD3	-0.42	0.081
19	APBRO4 × APBRD4	-1.73	-1.366
20	APBRO4 × APBRD5	-3.48	-3.178
21	APBRO5 × APBRD1	-4.66	-4.569
22	APBRO5 × APBRD2	-1.13	-0.523
23	APBRO5 × APBRD3	-1.55	-0.860
24	APBRO5 × APBRD4	-3.00	-2.451
25	APBRO5 × APBRD5	-3.04	-2.927

the present results are in support with the findings of Yokoyama (1979) and Singh & Hirobe (1964).

Low boil-off loss ratio improves reeling qualities

and it is manifested by dominant genes, while recessive genes act in the opposite direction (Gamo and Hirabayashi, 1984). In sericulturally advanced

Table 4. Performance of the silkworm hybrids

Hybrid	Pupa- tion Rate (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Reela- bility (%)	Raw silk (%)	Neat- ness (p)
APBRO1 x APBRD1	92.0	1.745	0.394	23.5	1018	84.0	19.3	92.4
APBRO1 x APBRD2	92.4	1.765	0.390	22.1	1048	84.5	19.0	92.4
APBRO1 x APBRD3	91.7	1.702	0.384	22.6	974	80.7	17.8	92.1
APBRO1 x APBRD4	90.8	1.746	0.382	21.9	883	79.4	18.2	91.5
APBRO1 x APBRD5	91.2	1.776	0.380	21.4	1039	84.6	18.0	92.4
APBRO2 x APBRD1	89.8	1.697	0.355	20.9	797	83.3	17.4	92.8
APBRO2 x APBRD2	90.8	1.757	0.374	21.3	1001	84.4	18.2	93.0
APBRO2 x APBRD3	91.2	1.712	0.366	21.4	997	85.2	18.5	91.5
APBRO2 x APBRD4	91.8	1.807	0.393	21.8	1027	84.6	18.2	90.7
APBRO2 x APBRD5	92.0	1.838	0.402	21.9	1040	84.8	18.6	92.4
APBRO3 x APBRD1	91.0	1.597	0.351	22.0	848	83.0	20.1	92.7
APBRO3 x APBRD2	91.5	1.607	0.348	21.7	989	83.8	18.9	90.8
APBRO3 x APBRD3	90.6	1.642	0.351	23.2	960	83.8	20.0	92.2
APBRO3 x APBRD4	92.4	1.630	0.370	23.3	885	83.9	20.1	94.2
APBRO3 x APBRD5	92.6	1.691	0.361	21.3	892	83.1	19.2	93.1
APBRO4 x APBRD1	89.8	1.741	0.360	21.8	940	84.9	18.8	92.0
APBRO4 x APBRD2	92.2	1.627	0.358	22.0	851	79.8	19.6	91.4
APBRO4 x APBRD3	91.9	1.704	0.376	22.1	916	80.4	20.1	90.2
APBRO4 x APBRD4	92.2	1.590	0.375	23.6	935	84.1	19.4	90.4
APBRO4 x APBRD5	92.4	1.735	0.380	21.5	943	86.0	19.5	92.3
APBRO5 x APBRD1	92.0	1.704	0.372	20.8	940	83.4	17.4	89.9
APBRO5 x APBRD2	92.4	1.727	0.367	20.6	926	86.2	17.9	90.5
APBRO5 x APBRD3	91.8	1.694	0.368	20.4	990	84.0	18.8	90.0
APBRO5 x APBRD4	92.5	1.768	0.385	22.0	881	86.0	18.4	92.8
APBRO5 x APBRD5	94.3	1.854	0.401	21.6	1020	88.4	19.8	93.4

Table 5. Evaluation Index values of silkworm hybrids for each of the trait

Hybrid	Pupa- tion Rate	Cocoon weight	Shell weight	Shell ratio	Filament length	Reel- ability	Raw silk	Neat- ness	Mean EI
APBRO1 x APBRD1	51.1	54.4	63.3	69.1	60.1	50.8	55.3	54.5	57.3
APBRO1 x APBRD2	69.3	57.2	60.5	52.8	64.3	53.1	51.8	54.5	57.9
APBRO1 x APBRD3	57.8	48.3	56.6	58.3	53.5	34.6	37.6	51.9	49.8
APBRO1 x APBRD4	50.3	54.5	55.3	50.2	40.3	28.3	42.3	46.7	46.0
APBRO1 x APBRD5	53.9	58.7	54.0	44.5	63.0	53.6	40.0	54.5	52.8
APBRO2 x APBRD1	37.1	47.6	37.9	38.9	27.7	47.4	32.9	58.0	40.9
APBRO2 x APBRD2	43.2	56.0	50.4	43.5	57.6	52.6	42.3	59.7	50.7
APBRO2 x APBRD3	43.2	49.6	45.0	44.4	56.9	56.5	45.9	46.7	48.5
APBRO2 x APBRD4	47.5	63.1	62.6	49.0	61.3	53.4	42.3	39.7	52.4
APBRO2 x APBRD5	50.0	67.5	68.0	49.9	63.2	54.7	47.1	54.5	56.9
APBRO3 x APBRD1	38.9	33.4	35.1	51.2	35.1	46.0	64.8	57.1	45.2
APBRO3 x APBRD2	44.6	34.8	33.4	47.6	55.8	49.9	50.6	40.6	44.7
APBRO3 x APBRD3	35.4	39.8	35.4	66.1	51.5	49.9	63.6	52.8	49.3
APBRO3 x APBRD4	54.3	38.1	47.6	66.8	40.6	50.2	64.8	70.2	54.1
APBRO3 x APBRD5	56.4	46.8	41.8	43.9	41.5	46.4	54.2	60.6	49.0
APBRO4 x APBRD1	26.1	53.8	41.1	49.8	48.6	55.1	49.4	51.0	46.9
APBRO4 x APBRD2	52.1	37.6	40.1	52.0	35.6	30.2	58.9	45.8	44.0
APBRO4 x APBRD3	48.9	48.6	51.5	52.4	45.1	33.1	64.8	35.3	47.5
APBRO4 x APBRD4	52.1	32.4	50.8	70.4	47.9	51.3	56.5	37.1	49.8
APBRO4 x APBRD5	54.6	52.9	54.0	45.3	49.0	60.6	57.7	53.6	53.5
APBRO5 x APBRD1	50.3	48.6	48.9	37.2	48.6	47.9	32.9	32.7	43.4
APBRO5 x APBRD2	54.6	51.9	45.7	35.3	46.5	61.4	38.8	38.0	46.5
APBRO5 x APBRD3	48.2	47.2	46.3	32.4	55.9	50.5	49.4	33.6	45.4
APBRO5 x APBRD4	55.3	57.6	57.2	51.7	40.0	60.4	44.7	58.0	53.1
APBRO5 x APBRD5	74.6	69.7	67.5	47.3	60.3	72.1	61.3	63.2	64.5

countries, the silkworm breeders have developed productive breeds / hybrids with low boil off ratio and quality silk (Gamo and Hirabayashi, 1983; Kurasawa, 1968). During the course of breeding, the boil off loss ratio with reference to cocoon shell has been given utmost importance along with other quantitative and qualitative traits (Gamo and Ichiba (1971); Harada, 1961; Mano *et al.*, 1988; Yokoyama, 1959). The analysed data on the performance of the parents and hybrids in the expression of boil-off loss ratio corroborate with earlier findings of Sidhu and Sonwalker (1969), Sinha *et al.*, 1992 and Sonwalker (1969). In the present study, less variation was observed among the oval (24.99%) and dumbbell (25.0%) lines regarding the boil-off loss ratio, whereas in hybrids it is 24.11%.

The phenomenon of heterosis and overdominance in conjunction with the expression of boil-off loss ratio analysed in the hybrids under present study facilitated procedures to identify the promising hybrids. Further, the more uniformity in the expression of this trait in hybrids than the parents is one of the desirable features to understand the genetic constitution of the hybrids for their commercial exploitation as evidenced by the mean values computed for boil-off loss ratio. For this trait, negative heterosis is desirable. For instance, high magnitude of negative heterosis was recorded in the combinations of APBRO5 x APBRD5, APBRO1 x APBRD1 and APBRO3 x APBRD4 which could be attributed to the higher mid parental values. The heterosis expressed is variable in different hybrids and these results are in confirmation with the findings of Gamo and Hirabayashi (1983). In certain hybrids, remarkably less heterosis was noticed and very often the hybrids were intermediate between parents for this trait. The result of the present study indicated the manifestation of heterosis in different hybrid combinations for this trait. Positive correlation that existed between boil-off loss ratio and cocoon shell weight of parental breeds clearly confirms the earlier findings of Gamo and Hirabayashi (1983). Improvement of boil-off loss ratio towards low value can be achieved through selection by choosing the

crossing types showing higher negative heterosis for this trait. Based on the hybrids performance, boil-off loss ratio, heterosis and heterobeltiosis and evaluation index, the hybrids APBRO5 x APBRD5 followed by APBRO1 x APBRD1, APBRO3 x APBRD4 were identified for further commercial exploitation.

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