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Studies on Boil-off Loss Ratio in the Cocoon Shells of Multivoltine × Bivoltine Hybrids of Silkworm, *Bombyx mori* L.

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The process of removal of gummy proteinous material sericin from silk is commonly called as degumming loss or boil-off loss ratio. In the present study, the boil-off loss ratio in the cocoon shells of twelve multivoltine × bivoltine hybrids and their parents were analysed. Inheritance pattern of boil-off loss ratio was analysed in crosses involving high and low boil-off loss parents, F_1s, F_2s and back-crosses by parent off spring regression analysis. Heterosis and heterobeltiosis was also analysed for this character. Highly significant ($P > 0.01$) variations were observed in eight out of ten multivoltine and two out of five bivoltine parents indicating the presence of genetic variation in the expression of boil-off loss ratio. Among F_1 hybrids, ten hybrids expressed significant ($P > 0.01$) variations when compared with control hybrid PM × NB_4D_2. Significant negative heterosis was expressed in three multi × bi hybrids *viz.*, BL67 × CSR_{101}, 96A × CSR_{19} and 96C × CSR_{19}, which is desirable for this character, whereas expression of heterobeltiosis was significant only with one hybrid, 96C × CSR_{18} in desired direction. Studies on inheritance pattern showed that the character is heritable and contribution percentage of female and male in the ratio of 50.9: 49.1 and it appears that both the parents are influencing in the expression of boil-off loss ratio in silkworm. Based on the overall performance and evaluation by multiple trait evaluation index and also considering the expression of the boil-off loss ratio three hybrids *viz.*, BL67 × CSR_{101}, 96A × CSR_{19} and 96C × CSR_{18} were found superior and recommended for commercial exploitation.

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Introduction

The domesticated mulberry silkworm, *Bombyx mori* L. spins a shell around its body for protection at the end of larval period by extruding proteinous material, silk bave. The silk bave is mainly composed of two protein substances namely fibroin and sericin. Fibroin forms inner core of the silk thread representing 70 to 80% parts while sericin forms 20 to 30% parts as outer layer of the silk filament. Apart from these two constituents, a small amount of fats, resin, mineral matter, waxy substance and colouring matter is also present which forms around 2 to 3% parts in the silk. The fibroin of silk is insoluble in alkaline hot water whereas the gummy protein of silk, sericin is easily soluble in boiling alkaline soap solution due to loose molecular structure and rich hydrophobic amino acids (Kannan, 1986). The process of elimination/ removal of gummy substance (sericin) from silk are called as degumming of silk. The degumming loss/boil-off loss has been considered as one of the important parameters during the course of breeding by the silkworm breeders of sericulturally advanced countries. Boil-off loss percentage is high in cocoon shells when compared to raw silk. Degumming loss percentage was high in multivoltine breeds when compared to bivoltine breeds (Siddu and Sonwalkar, 1969) and high in cocoon shells with floss when compared to shells with out floss (Sonwalkar, 1969). Boil-off loss ratio varies significantly among different silkworm strains and the same is adversely influenced by environmental conditions.

Degumming loss varies in different silkworm strains which may in turn effects weaving industry. Very little information is available in the boil-off ratio of silk of cocoon shells of newly evolved multivoltine strains and their F_1 hybrids. In the present study an attempt has been made to study boil-off loss ratio of cocoon shell of some multivoltine breeds and their F_1 hybrids of silkworm.

Materials and Methods

Ten multivoltine silkworm breeds *viz.*, Pure Mysore, C.Nichi, BL22, BL23, BL24, BL43, BL67, 96A, 96C and 96E; six bivoltine breeds *viz.*, NB_4D_2, CSR_5, CSR_{18}, CSR_{19} CSR_{101} and PC (g) and twelve F_1 hybrids were reared in completely ramdomized design (CRD) in three different seasons of the year 2001 following standard rearing method suggested by Krishnaswami (1978). Data was recorded for fecundity, larval span, yield/10,000 larvae both by number and weight, cocoon weight, cocoon shell weight, shell ratio percentage. To study post cocoon parameters, 100 numbers green cocoons were reeled in reeling and fibre technology division of CSRTI, Mysore by utilizing multi end reeling machine and recorded filament length, denier, reelability percentage, raw silk percentage and neatness.

After sex separation and cocoon assessment, 30 cocoon shells each from male and female sexes were taken at random. The cocoon shells were replicated into three having ten shell in each group. Degumming of cocoon shells was carried out by boiling them in alkaline soap solution.

The degumming process was carried out directly by boiling cocoon shells in alkaline soap solution on LPG, gas stove. Two step boiling method (Basavaraja *et al.*, 2000) was followed for degumming of cocoon shells. Sample of 10 cocoon shells taken in a craft paper cover and dried in automatic electronic woven for 3 hrs at 105°C and then for 5 hrs at 60°C. The craft paper covers removed from woven and kept in desicator for one hour for cooling and measured dried weight of cocoon shells.

First degumming: Dried cocoon shells from each craft cover are transferred into porous cloth bagsand tied with twine thread. Four liters of alkaline soap solution (1 g of sodium carbonate and 2 g of marusel soap in one liter of water) boiled indirectly in a stainless steel container. When the solution reached the boiling point (> 95°C), 20 cocoon shell sample bags were immerced completely in boiling solution and kept tightly covered for 40 min. At every 10 min interval the sample bags were turned up and down for uniform and effective degumming. The bags were then removed and washed by rubbing with hands in tap water and dehydrated for two minutes in washing machine.

Second degumming: In another stainless steel container, four liters of alkaline soap solution (1 g of sodium carbonate and 2 g of marusel soap in one liter of water) was prepared and kept ready at boiling point (> 95°C) to transfer the sample bags after first boiling. Immediately after dehydration in washing machine, 20 sample bags were transferred to the boiling alkaline soap solution. Second degumming was carried out for another 40 min with stirring at 10 min interval.

Rinsing: Immediately after second degumming, the sample bags containing degummed shells washed by rubbing with hands in tap water and dehydrated in washing machine for two minutes. Then the bags were transferred into an already prepared 0.5% sodium carbonate solution at 50°C for five min and rinsed well.

Washing: After rinsing in 0.5% sodium carbonate solution, the sample bags with cocoon shells were washed thoroughly (for 15 min) in running water and dehydrated in washing machine for five min.

Drying and weighing: The degummed cocoon shells were transferred in to the new craft paper covers and dried for 3 hrs at 105°C and then at 60°C for 5 hrs. The dried craft covers were then transferred to the desicator for one hour for absorption of excess moisture before taking dry weight of the degummed cocoon shells.

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

$$\text{Boil-off loss ratio} = \frac{\text{Initial dry weight of cocoon shells} - \text{Final dry weight of degummed shells}}{\text{Initial dry weight of cocoon shells}} \times 100$$

Inheritance studies were carried out by crossing multivoltine breeds in the following pattern: High and low parents; F_1s (High × Low; Low × High) F_2s (High × Low; Low × High) and reciprocals (High × Low) × High, (Low × High) × High; (High × Low) × Low; (Low × High) × Low) are analysed boil-off loss ratio. Inheritance pattern was analysed by Parent offspring regression analysis.

Heterosis over mid parent and better parent values were calculated by using the following formulae:

$$\text{Heterosis(\%) over MPV} = \frac{F_1 - MPV}{MPV} \times 100$$

$$\text{Heterosis(\%) over BPV} = \frac{F_1 - BPV}{BPV} \times 100$$

Multiple trait evaluation index method (Mano *et al.*, 1993) was employed for ranking of hybrids following the formula:

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

Where,
A = Value obtained for a particular trait of a particular hybrid,
B = Mean value of the particular trait of all the hybrids,
C = Standard deviation, 10 = Standard unit and, 50 = Fixed value.

Results

The boil-off loss ratio percentage in the cocoon shells of the parental multivoltine, bivoltine strains and F_1 hybrids are presented in Table 1. A great deal of variation was observed in the boil-off loss ratio of multivoltine breeds. It ranged from 23.15% to 27.92% in different breeds. Lowest boil-off loss was observed in the breed C.Nichi (23.15%) whereas highest value was exhibited by BL22 (27.92%). Different multivoltine breeds exhibited highly significant differences ($P > 0.01$) when compared with the popular Pure Mysore except BL43. The boil-off loss ratio in bivoltine breeds utilized as male parent ranged from 24.96% (CSR_{18}) to 27.42% [PC (g)]. The breeds CSR_5 and CSR_{18} exhibited non significant differences when compared to control NB_4D_2.

Mean boil-off loss ratio percentage of twelve F_1 hybrids are also presented in Table 1. It ranged from 22.62% (96C × CSR_{18}) to 27.50% (PM × NB_4D_2). Highly significant differences were observed in ten out of twelve F_1 hybrids in the boil-off loss ratio of multivoltine × bivoltine hybrids except BL43 × NB_4D_2 and BL67 × PC (g) where non- significant differences were observed.

Studies on inheritance pattern of boil-off ratio has been presented in Table 2. It is clear from the data that the boil-off loss ratio in high parent and back- crossed with high parent expressed higher value where as the other crosses expressed lower values.

Heterosis percentage over mid parent value and better parent values of boil-off loss ratio has been presented in Table 3. Lowest negative heterotic values for this trait was observed in the hybrid 96C × CSR_{18} (–6.14%) followed by 96A × CSR_{19} (–4.80%) and BL67 × CSR_{101} (–3.68%). Highest positive heterotic values were observed in the hybrids BL67 × PC (g) (5.57%) followed by PM × NB_4D_2 (3.58%) and BL43 × NB_4D_2 (1.25%). Heterobeltiosis for boil-off loss ratio in different hybrids ranged from 11.57% [BL67 × PC (g)] to –9.38 (96C × CSR_{18}). Out of twelve F_1 hybrids, 96C × CSR_{18} have registered significant ($p < 0.05$) negative heterobeltiosis whereas three hybrids *viz*., PM × C.Nichi, BL67 × PC (g) and PM × NB_4D_2 have expressed significant positive heterosis for this trait.

Mean performance of twelve F_1 hybrids are presented in Table 4. It is clear from the data that highly significant differences were observed among twelve hybrids for the characters cocoon yield/10,000 larvae by weight, cocoon weight, shell weight, shell ratio, filament length, denier, reelability percentage and neatness when compared with the control hybrid, PM × NB_4D_2. Mean performance of the hybrid data were subjected to multiple trait evaluation index for selection of promosing hybrid combinations. Based on the average evaluation index scored, top three hybrids *viz*., BL67 × CSR CSR_{101}, 96A × CSR CSR_{19} and 96C × CSR_{18} were selected as promising.

Table 1. Mean boil-off loss ratio of parental multivaltine, bivoltine breeds and multivoltine × bivoltine F_1 hybrids of silkworm (Mean of 3 seasons)

Sl. no.	Breed	Boil-off ratio
Multivoltine		
1	C.Nichi	23.15**
2	BL22	27.92 NS
3	BL23	25.76**
4	BL24	26.21**
5	BL43	26.93 NS
6	BL67	24.63**
7	96A	23.95**
8	96C	23.24**
9	96E	25.28**
10	Pure Mysore(Control)	27.56
Bivoltine breeds		
1	CSR_5	25.32 NS
2	CSR_{18}	24.96 NS
3	CSR_{19}	26.36*
4	CSR_{101}	26.46*
5	PC(g)	27.42**
6	NB_4D_2 (Control)	25.53
Multivoltine × bivoltine F1 hybrids		
1	PM × C. Nichi	25.58**
2	BL23 × NB_4D_2	25.77**
3	BL24 × NB_4D_2	26.01**
4	BL43 × NB_4D_2	26.56 NS
5	BL67 × CSR_5	24.22**
6	BL67 × CSR_{101}	24.61**
7	BL67 × NB_4D_2	24.95**
8	96A × CSR_{19}	24.00**
9	96C × CSR_{18}	22.62**
10	96E × CSR_{19}	25.27**
11	BL67 × PC(g)	27.48 NS
12	PM × NB_4D_2 (Control)	27.50

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

Table 2. Inheritance pattern of boil-off loss ratio in multivoltine silkworm

	Parent / Cross	Boil-off ratio	b value	R^2
High	BL22	27.92	-0.750	0.43
Low	C.Nichi	22.63	0.285	0.63
F_1 hybrids				
High x Low	BL22 x C.Nichi	24.20	-0.500	0.05
Low x High	C.Nichi x BL22	24.72	0.290	0.18
F_2 Generation				
High x Low	BL22 x C.Nichi	24.78	1.800	0.99
Low x High	C.Nichi x BL22	24.35	-0.589	0.99
Reciprocal hybrids				
(High x Low) x High	(BL22 x C.Nichi) x BL22	27.55	0.430	0.25
(Low x High) x High	(C.Nichi x BL22) x BL22	25.35	0.214	1.00
(High x Low) x Low	(BL22 x C.Nichi) x C.Nichi	24.15	0.101	0.14
(Low x High) x Low	(C.Nichi x BL22) xC.Nichi	25.22	0.918	0.24

Table 3. Heterosis (%) over mid parent (MPV) and heterobeltiosis (%) bettter Parent (BPV) of boiloff loss ratio in multi voltine x bivoltine hybrids

Sl. No.	Hybrid	Heterosis over MPV	Heterosis over BPV
1	PM x C. Nichi	-1.04	5.92*
2	BL23 x NB_4D_2	0.47	0.94
3	BL24 x NB_4D_2	0.54	1.88
4	BL43 x NB_4D_2	1.25	4.03
5	BL67 x CSR_5	-3.04	-1.66
6	BL67 x CSR_{101}	-3.68*	-0.08
7	BL67 x NB_4D_2	-0.52	1.30
8	96A x CSR_{19}	-4.80*	0.21
9	96C x CSR_{18}	-6.14*	-9.38*
10	96E x CSR_{19}	-2.32	-0.04
11	BL67 x PC(g)	5.57*	11.57*
12	PM x NB_4D_2 (Control)	3.58*	7.72*

*,** Significant at 5% level.

Discussion

The process of removal of gummy proteinous material sericin from silk is commonly called as degumming loss or boil-off loss ratio. It is synonymous to the scouring process used for the purification of other fibres like cotton and wool. Boil-off loss ratio in silkworm is heritable and silkworm breeders in sericulturally advanced countries have evolved silkworm breeds/hybrids with low boil-off ratio (Gamo and Hirabayashi, 1984). In the present study, it is observed that variations was observed in the expression of boil off ratio of parents both multivoltine and bivoltine breeds. The trait in multivoltine and bivoltine breeds ranged from 27.92% (BL 22) to 23.15% (C.Nichi) and 27.42% (PC, g) to 24.96% (CSR_{18}) respectively. Variations in the expression of this trait in different breeds may be due to their genetic diversity and origin. Sinha et *al.* (1992) reported significant variation for moisture percentage, boil-off loss and total minerals in the cocoon shells of multivoltine races *viz.*, N, G, N x G, S15 and Sonwalkar (1969) studied boil-off ratio in multivoltine breeds, *viz.*, Pure Mysore, Mysore Princess, Kolar Gold and Kollegal Jawan and reported significant differences in the boil-off ratio between breeds and between cocoon shells with floss and without floss. The present findings corroborate the findings of the above authors. Boil-off loss ratio in bivoltine breeds used as male parents in the present study show narrow difference in the expression of the trait, which may be attributed to their origin. Recently, Basavaraja *et al.* (2000) have reported variations in the expression of boil-off loss ratio in twelve bivoltine breeds of silkworm. Inheritanc studies on boil-off loss ratio indicated that the trait is heritable and it appears both the parents are influencing for the expression of this trait.

Expression of boil-off ratio in multivoltine x bivoltine hybrids have been presented in Table 1. It is clear from the data that a wide range of variation exists in the expression of this trait. Highest value was recorded in the hybrid PM x NB_4D_2 (27.50%), where as lowest value (22.62%) was recorded in 96C x CSR_{18}. BL43 x NB_4D_2 expressed non-significant differences when compared to control, while the other hybrids showed significant difference. Gamo and Hirabayashi (1984) emphasised that lower boil-off ratio is manifested by dominant genes, while recessive genes act towards opposite direction. They also opined that boil-off loss ratio towards low value can be achieved by its selec-

Table 4. Mean performance of few multivoltine x bivoltine hybrids of silkworm (Mean of 3 seasons)

Sl. no.	Hybrid	Fecundity	Larval span (hrs)	Yield/10,000 larvae by no.	by wt (kg)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Denier	Reelability	Raw silk (%)	Neatness	Average Evaluation Index value
1	PM ×C.Nichi	450	480	8967	13.20	1.475	20.0	13.4	512	2.08	87.7	10.7	84.3	34
2	BL23 × NB_4D_2	468	509	8917	16.30	1.716	31.8	18.5	750	2.75	86.0	12.5	86.0	36
3	BL24 × NB_4D_2	503	505	8850	14.50	1.680	31.4	18.7	835	2.89	87.7	13.9	89.7	51
4	BL43 × NB_4D_2	512	510	8789	15.80	1.738	32.3	18.6	810	3.04	85.0	13.3	87.3	41
5	BL67 × CSR_5	496	475	8811	15.80	1.776	35.7	19.8	1007	2.78	88.3	14.6	91.3	52
6	BL67 ×CSR_{101}	482	480	9245	16.70	1.796	34.7	19.3	932	2.84	88.7	15.3	91.6	54* I
7	BL67 × NB_4D_2	493	488	8789	18.00	1.982	37.6	18.9	925	2.81	86.8	14.2	91.3	52
8	96A × CSR_{19}	491	480	9384	19.20	2.026	39.6	20.3	925	2.95	87.7	14.4	90.0	53* II
9	96C × CSR_{18}	486	498	9167	13.60	1.545	34.2	22.2	1018	2.72	85.7	15.8	91.3	53* III
10	96E × CSR_{19}	465	513	8798	15.10	1.731	36.1	20.9	1017	2.85	83.1	16.5	92.0	52
11	BL67 × PC(g)	508	488	8920	14.90	1.637	34.6	21.2	992	2.88	85.5	15.8	91.3	52
12	PM × NB_4D_2 (Control)	432	519	8642	13.70	1.687	31.3	18.5	747	2.57	85.0	12.2	87.0	40
CD at 5%		----	-----	-----	2.10	0.14	0.003	1.30	48.0	0.14	------	0.79	1.25	
1%		6.90	4.56	4.04	7.95	4.77	0.46	2.25	3.26	2.92	3.24	3.32	0.82	

*, Selected hybrids

tion and by the finding of the crossing types showing higher combining ability. Basavaraja *et al.* (2000) have reported existence of significant positive correlation between boil-off loss ratio and cocoon shells in parental bivoltine breeds. Huang (1989) have reported presence of marked positive correlation in the Chinese bivoltine strains cocoon shell sericin percent with cocoon shell weight, shell ratio and filament length. The present findings are in conformity with the findings of others.

Studies on hybrid vigour over MPV and BPV has been presented in Table 3. Three hybrids *viz.*, BL67 × CSR_{101}, 96A × CSR_{19}, 96C × CSR_{18} expressed significant negative heterosis over MPV, which is desirable for this trait, whereas 96C × CSR_{18} alone expressed significant negative heterobeltiosis. Similar type of results were reported by Gamo and Hirabayashi (1984) and Basavaraja *et al.* (2000) opined improvement of boil-off loss ratio towards lower value can be achieved through selection by crossing types showing negative heterosis for this trait.

Based on the expression of boil-off loss ratio and mean performance of twelve hybrids in different seasons, three hybrids *viz.*, BL67 × CSR CSR_{101}, 96A × CSR_{19}, 96C × CSR_{18} have been selected for commercial exploitation.

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