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Evaluation of F_1 population of tropical tasar silkworm *Antheraea mylitta* D (Lepidoptera: Saturniid) through cocoon and post cocoon parameters reared on different food plants

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Abstract
The quality and quantity of the silk produced by phytophagous silkworms are invariably influenced by the quality of the food and genetic background of the silkworm. In the present study four F_1 populations of tasar silkworm *Antheraea mylitta* were prepared and reared on different primary host plants along with its parental populations. Subsequent to harvest of cocoons, cocoon and post cocoon characters were analyzed and compared with different groups. The results indicate that F1 populations recorded significantly ($p<0.001$) higher values in some of the parameters studied. Amongst the food plants, *Terminalia* fed silkworm cocoons recorded higher average cocoon weight (12.89 ±1.83) in F_1 cross of Daba and Laria. Shell weight was higher in the Sal fed tasar silkworm cocoons and higher shell weight was in F_1 cross with Sal based ecoraces Laria x Raily (2.12±2.4g). Similarly, the post cocoon characters such as filament length (1097mts.) and other silk quality parameters recorded better values in the Sal fed F1 hybrids compared to the parental populations. The study indicates there is an apparent influence of the host plants as well as the silkworm breed on the quality of the cocoon and subsequent silk quantity and the quality.

Keywords: *Antheraea mylitta,* host plant, post cocoon characters, reciprocal cross, F_1 hybrid, Daba, Raily, Laria

1. Introduction

Antheraea mylitta D has rich genetic resources, with 44 ecoraces distributed in different ecological systems of which very few are being exploited for commercial silk production [1]. Tasar silkworms are polyphagous, feeds on wide range of food plants with three primary and more than dozen secondary food plants [2]. The quality of leaf plays an important role in the nutrition of silkworm and in turn cocoons and silk production. There is an apparent influence of quality of leaf on the expression of phenotypic characters especially the cocoon and post cocoon characters [3]. Cocoon and shell weights are the major traits evaluated for productivity in sericulture and have been used for more than half a century. Cocoon weight is an important commercial characteristic used to determine approximately the amount of raw silk that can be obtained. Shell weight gives a better estimate of the total silk on commercial point of view.

In sericulture, exploitation of hybrid vigour is imperative for higher productivity of silk and other quantitative traits. The breeding approaches to envisage the hybrid vigour is by combining genetically divergent silkworm parents or to use wild relatives to produce hybrids [4, 5]. Evolution of improved breeds through breeding of hybrids is well-documented in *B. mori*. Hybrid vigour is at its bests in F_1 hybrid which decreases gradually in subsequent generations and the phenomenon of hybrid vigour disappears in about 14 generations in silkworm [6]. The selection of parents is always vital and hence wild ecoraces which proven its vigority, sustainability in different conditions are choice of the breeders to enhance the adaptability, survivability and productivity of silkworm breeds. The selection of the parent races should be precise as per commercial requirement and traits of productivity [7, 8]. Crossing among genetically divergent ecoraces for introgression of gene/traits of commercially viable characters from semi-domesticated with wild relative donor parent establishes promising outcome [9, 10]. F1 (single cross) hybrids are most commonly used for commercial cocoon production because they represent high heterosis for most of the economic characters [11]. Hybridization studies were also explored for cocoon weight, shell weight, silk ratio, fecundity, and larval weight in *A. mylitta* [12]. Nine inbred lines – GB1, GB2, GB8, GB12, GB13, GE1, GE3, GE4 and GF4, were crossed with tester Daba [13] and highest heterosis with regard to hatching percentage, cocoon weight and shell weight were recorded in cross combinations – GB12 x Daba, GE3 x Daba and GB13 x Daba, respectively. It observed [14] that characters- shell ratio, yield/dfl and fecundity were found important traits for selection studies and it also indicated predominantly higher SCA values for fecundity and shell weight. It was observed that size deviation is a racial character, lesser size deviation increases the reelability percentage and raw silk recovery as long filament of fine denier will have less breaks in the reeling process [15]. Improvement of breeds necessarily means selection of desirable genes in appropriate combinations, which contribute to the overall genetic worth of the population. With respect to the economic value, the focus should be on all the genes affecting the traits thereby contributing to the viability and productivity. Therefore, selection of hybrid combinations emphasizes the need to organize the genetic material in a way that helps to improve the manifestation of commercially important traits [16]. The increased productive characters and silk quality parameters in the F_1 populations obtained through the crossing of different selective parents of semi-domesticated races with the wild populations were observed recently in tropical tasar silkworm [17, 18].

Based on the above information, in the present study, crossing of wild Sal based ecoraces of tropical tasar silkworm (Laria & Raily) with semi-domesticated race (Daba) has been explored to examine the heterosis effect in the F_1 hybrids. Since the cocoon and silk filaments are the final products of the sericulture industry, evaluation of the heterosis effect in the F_1 hybrids considering the cocoon and post-cocoon characters would be most desirable and also the influence of different host plants on these quantitative and qualitative characters were examined.

2. Materials and Methods

2.1 Collection of cocoons of parent population for cross breeding

Daba semi-domesticated cocoons were collected from Silkworm breeding & Genetics section, Central Tasar Research &Training Institute (CTR&TI), Ranchi. Nature grown cocoons of Laria was collected from the Sal forest in the Peterbar area of Jharkhand State and Raily cocoons were collected from its natural eco-niches *ie.*, Sal forest of Bastar District of Chhattisgarh State. The parent cocoon characters were recorded such as cocoon wt., shell wt. and silk ratio in all the three races. The cocoons were preserved separately in separate out-door cages specified for preservation of cocoons at the Silkworm breeding & Genetics Grainage area of CTR&TI, Ranchi till the moth emerges.

Table 1 represents the general cocoon features of the parent populations of different ecoraces selected in the present study for the preparation of F1 hybrids through reciprocal cross. Raily cocoons recorded higher cocoon traits (C Wt.:13.30g, S Wt.: 2.60g, Silk Ratio: 19.54%) compared to semi-domesticated Daba and Laria (wild) with higher deviation in the data.

Table 1: Cocoon features of parent populations collected from different sources

	Daba			Laria			Raily		
	Cocoon Weight (g)	**Shell Weight (g)**	**Silk ratio (%)**	**Cocoon Weight (g)**	**Shell Weight (g)**	**Silk ratio (%)**	**Cocoon Weight (g)**	**Shell Weight (g)**	**Silk ratio (%)**
Mean	12.09	1.45	11.99	7.34	0.89	12.12	13.30	2.60	19.54
SD	2.32	0.26	1.25	1.51	0.28	0.65	2.99	0.44	1.38
Min	7.18	1.01	11.80	4.41	0.43	09.75	8.84	1.92	16.35
Max	16.15	2.03	12.57	12.34	1.61	13.05	19.89	3.65	22.6

2.2 Preparation of reciprocal crosses and Rearing

When moth emergence started, the moths were collected according to the plan to prepare reciprocal crosses such as Laria X Daba, Daba X Laria, Laria X Raily, Raily X Laria and Parental crosses also prepared Daba x Daba, Laria x Laria and Raily x Raily. These crosses were prepared through induced crossing (hand coupling) except in semi-domesticated Daba x Daba. The each batch of coupling (mating) was maintained for 6 hours. After decoupling, mother moths were collected and kept for oviposition for 3 days as per the standard procedure. Silkworms of different reciprocal crosses on the day of hatching were brushed separately on three food plants such as *Terminalia arjuna* (Arjun), *T. tomentosa* (Asan) and *Shorea robusta* (Sal). The complete out-door rearing was conducted as per the standard tasar silkworm rearing practices. After the harvest of the cocoons, different batch cocoons were sorted to discard damaged, melt and flimsy cocoons. The good cocoons were selected for further quantitative as well as qualitative traits analysis.

2.3 Assessment of quantitative cocoon and Post-cocoon characters

About 100 good cocoons were randomly selected from each groups separately, the quantitative character of cocoons such as Cocoon wt., shell wt. and silk% was recorded in different F1 groups and parent lines. About 300 good cocoons from each group were randomly selected and used for the assessment of post-cocoon characters such as filament length (mts), Non-breakable filament length (NBFL-mts.), filament denier, reelability (%) and Silk recovery (%).

2.4 Statistical Analysis

One-way analysis of variance was used to test the significance of differences between the mean values of independent observations. Comparisons were performed with Duncan's Multiple Range Test (DMRT) to find significant differences between the batches. Differences were considered significant at $p < 0.05$ [19].

3. Results

The F1 hybrids were prepared through reciprocal crosses of semi-domesticated tasar race with the wild ecoraces and evaluated their cocoon and post-cocoon performance on different host plants. The results obtained are compiled as follows;

3.1 Quantitative cocoon characters

1. Cocoon weight: Cocoon wt. was found significant difference at $p<0.001$ among the different races/groups higher cocoon wt. was recorded in the Laria x Daba (12.62 ±2.92g) followed by in Raily (12.50 ±3.15g) and lowest cocoon wt. was recorded in Laria reared on Sal plant (10.27 ±1.89g). The F1 populations recorded better in cocoon weights compared to parent populations. It was observed that the tasar silkworms reared on *Terminalia* food plants that to those silkworms which fed on Asan leaves recorded more cocoon weight.

2. **Shell weight:** shell weights were recorded in contrast to cocoon weight higher was recorded in the hybrid combination Laria x Raily (2.12±1.89g) compared other races/combinations. Sal reared silkworms gained more shell weight compared to cocoons harvested from *Terminalia* food plants. Among *Terminalia* food plants, cocoons grown in Arjun plants found better than Asan. Similar to cocoon wt., hybrid combinations recorded higher shell weights than the parent populations.
3. **Shell ratio:** corresponding to shell wt., the shell ratio was also found higher in the hybrid combinations reared on Sal food plants. Higher SR was recorded with Laria x Raily (17.29±1.27g) compared to the parent populations.

3.2 Post-Cocoon characters

In a concurrence to cocoon characters, post cocoon characters were also found better in the F1 populations. However, tasar silkworms reared on Sal plants owe better post cocoon characters. It was observed a highly significant (@ $p<0.001$) differences among the different races and F1 populations in the almost all the post cocoon characters studied. However, no significant difference found in the characters compared with different food plants. The F1, Laria X Raily cocoons grown on Sal found higher filament length (1096.67± 107.74 mts.) with better NBFL (338.33±40.2), reduced denier (9.62), average reelability (32.65%) and high silk recovery (66.67%) followed by Raily cocoons harvested from Sal. Laria cocoons recorded lower filament length (600 to 612 mtrs), lower NBFL (188.33±28.43 mtrs.), better denier (9.10) with lesser reelability (23.39 ± 2.75%) and silk recovery (49.69%). Over all the performance of the F1 hybrids was better when compared to the mid parent value in most of the post-cocoon parameters studied. More than 25-30% increase in the silk recovery was recorded in the reciprocal crosses of Laria and Raily cocoons (Table 2).

4. Discussion

In silkworms, the performance of a race or a breed is mainly dependent on the combined action of hereditary potential of its population and the extent to which such potential is permitted to express in the environment to which it is exposed. The performance of different ecoraces of tropical tasar silkworm and its hybrid combinations reared on different host plants showed significant difference in expression of cocoon and post-cocoon characters in the present study is in conformity with the observations of several workers [20-25]. This is largely due to the variable gene frequencies at different loci in different silkworm races which make them to respond differently to changing environmental conditions and food [26, 27]. The cocoon characters are important components, which determine the overall performance of the silkworm and also important for subsequent silk fiber quality. Various factors such as genetic background of silkworm, food quality, environment and etc., influence the economic traits of the silkworm [28]. The significant variations among the different groups in respect of silk filament length, higher values were recorded in the hybrid populations suggest the higher impact of hybridization on those traits compared to other characters which were non- significant [20]. The cocoon characters and post-cocoon characters among F1 of different combinations features the compatibility and genetic capability of these crosses in expression of higher cocoon traits compared to the Daba (ruling tasar silkworm race). Significant difference in cocoon characteristics was observed for the tasar silkworms reared on different food plant species. Comparatively, higher larval weights resulted higher cocoon weights in *T. tomentosa*, *T. arjuna* and *T. belerica*, which might be due to better rate of quantity of food in-taken, digested and assimilated [29, 30]. Dietary changes in the silkworm influence on the cocoon characters and on post cocoon characters such as quantity of the silk filament and denier [31]. The primary food plants are most suitable for the tasar silkworms compared to the secondary food plants in the silk outcome [32]. It is also understood that crossing the commercially exploited domesticated silkworms with its wild counterparts would always yield better [33]. While, deterioration in some characters may also be occurred when crossing distantly related silkworms due to incompatibility as observed in present study Daba x Laria for commercial cocoon traits. Laria and Raily are wild ecoraces and adapted by Sal as primary food plants so this would be the factor for the compatibility and better performance with respect to the cocoon traits. The genotype-environment interaction has a significant role in expressing the commercial cocoon traits in tropical silkworms [34, 35].

5. Conclusion

The better performance of the F1 populations in commercially important traits, reared on different host plants in the present study gives an insight in to the selection of parents for hybridization on one hand and the impact of food plants on expression of higher commercial characters in specific traits on the other. Also it reiterates the prospects of developing hybrid tasar silkworms obtained through crossing between Sal based wild ecoraces and rearing these silkworms on Sal to harvest cocoons with better silk quality and quantity.

Table 2: Performance F1 populations and its parents on different cocoon and post cocoon parameters reared on three primary food plants

Ecorace/ combination	Food Plant	Cocoon Weight (g)	Shell Wt. (g)	Shell Ratio (%)	Filament length (mts.)	NBFL (mts.)	Denier	Reelability (%)	Silk Recovery (%)
Daba	Arjun	12.17 ± 2.61	1.82 ± 0.35	14.92 ± 0.32	986.33 ± 104.64	232.67 ± 22.81	9.87 ±0.19	27.47 ±5.57	57.15 ±7.22
	Asan	12.20 ± 2.60	1.74 ± 0.30	14.33 ± 0.55	951.67 ±35.12	215.00 ±20.00	9.92 ±0.23	26.87 ±6.21	56.98 ±7.34
Laria	Arjun	10.63 ± 2.41	1.58 ± 0.25	14.81 ± 0.76	605.00 ±20.00	188.33 ± 28.43	9.15 ±0.38	28.65 ±3.53	52.15 ±6.29
	Asan	10.71 ± 2.44	1.52 ± 0.14	14.23 ±0.71	600.00 ±30.00	197.00 ± 11.53	9.50 ±0.31	31.50 6.26	51.51 ±5.79
	Sal	10.27	1.68	16.19	612.33	205.00	9.01	23.39	49.60

		± 1.89	± 0.24	± 1.59	± 31.09	± 13.23	± 0.06	± 2.75	± 6.10
Raily	Arjun	12.19 ± 2.96	1.88 ± 0.33	15.44 ± 1.19	835.00 ± 35.34	215.00 ± 22.34	10.35 ±0.15	31.11 ±6.84	53.50 ±4.39
	Asan	12.48 ± 2.94	1.80 ± 0.30	14.45 ± 1.18	822.23 ± 34.12	225.00 ±30.00	10.28 ±0.19	32.50 7.54	50.81 ±4.22
	Sal	12.50 ± 3.15	2.08 ± 0.52	16.64 ± 1.27	1021.00 ± 171.41	228.33 ±30.14	10.53 ± 0.28	22.18 ± 2.02	52.13 ± 4.31
Laria X Daba	Arjun	12.39 ± 3.00	1.94 ± 0.41	15.65 ± 1.50	938.33 ± 102.14	213.00 ± 24.02	9.50 ±0.30	38.50 ±7.21	58.45 ±8.55
	Asan	12.62 ± 2.92	1.83 ± 0.34	14.50 ± 0.96	820.00 ± 45.21	275.33 ±42.25	9050 ±0.22	30.50 ±7.11	57.80 ±7.63
	Sal	10.90 ± 2.30	1.79 ± 0.34	16.60 ±1.02	811.67 ± 47.3	225.33 ± 27.3	9.32 ± 0.21	27.50 ± 6.15	56.67 ± 5.84
Daba X Laria	Arjun	12.31 ± 2.60	1.80 ± 0.22	14.66 ±1.59	790.00 ±20.22	220.00 ±27.84	9.80 ±0.35	36.50 ±4.58	49.53 ±7.05
	Asan	12.36 ± 2.58	1.73 ± 0.29	14.05 ± 1.39	794.67 20.03	235.00 ±14.18	10.01 ±0.18	34.00 ±4.36	50.12 ±6.80
	Sal	11.13 ± 2.49	1.74 ± 0.30	15.60 ± 0.71	798.00 ± 20.66	220.00 ±27.84	9.67 ± 0.27	29.86 ± 1.00	57.00 ± 6.21
Raily X Laria	Arjun	12.140 ± 2.59	1.79 ± 0.27	14.71 ±1.58	815.00 66.14	350.00 ±55.68	10.20 ±0.18	38.50 ±3.61	53.02 ±6.21
	Asan	12.240 ± 2.64	1.68 ± 0.23	13.78 ±1.10	790.00 ± 52.92	335.00 ±58.95	10.35 ±0.20	32.80 ±3.24	52.13 ±5.18
	Sal	12.19 ± 3.21	1.97 ± 0.33	16.18 ± 1.61	887.33 ± 58.53	371.0 ± 49.43	10.20 ± 0.22	34.42 ± 3.69	63.69 ± 9.19
Laria X Raily	Arjun	12.05 ± 3.09	1.82 ± 0.30	15.12 ±1.10	835.00 57.66	311.67 ± 28.43	10.30 ±0.18	36.45 ±4.62	62.53 ±7.27
	Asan	12.10 ± 3.17	1.75 ± 0.23	14.47 ±1.56	810.00 ±35.00	310.00 ±30.41	10.09 ±0.15	30.50 ±2.73	60.80 ±4.78
	Sal	12.29 ± 3.10	2.12 ± 0.40	17.29 ± 1.27	1096.67 ± 107.74	338.33 ± 40.22	9.62 ± 0.28	32.65 ± 3.88	66.67 ± 4.33
P Value	Replicate	0.00***	0.00***	0.00***	0.117	0.1027	0.3630	0.0006***	0.0214*
	Races	0.00***	0.033**	0.3207	0.00***	0.00***	0.00***	0.0188*	0.0160*
	Food plant	0.7013	0.0428*	0.0013**	0.0634	0.3660	0.3658	0.0550	0.6246

Significance Levels * = <.05, ** = <.01 & *** = <.001

Table 2b: Summary of ANOVA representing the Mean Sum of Squares for the parameter over Races and food plants

	DF	Cocoon wt.	Shell wt.	SR	Filament length	NBFL	Denier	Reelability	Silk Recovery
Replicates	2	104.344***	0.971***	13.827***	6347.167	2394.1	0.061	183.078	149.6
Races	7	2.303***	0.068**	0.521	73892.7***	17650***	0.886***	57.208*	108.86
food plant	1	0.030	0.070*	5.472**	9997.7	814.88	0.049	73.471	8.202
race x food plant	6	0.035	0.001	0.043	2499.16	1070.54	0.053	24.9	1.7
Error (B)	26	0.202	0.015	0.422	2658.8	962.559	0.057	18.201	33.45
Total	41	5.561	0.069	1.158	13418.8	3486.7	0.178	34.278	44.9
General mean		12.042	1.763	14.646	813.8	251.64	9.915	32.561	54.75
C.V		3.733	7.028	4.434	6.336	12.329	2.418	13.102	10.56

Significance Levels * = <.05, ** = <.01 & *** = <.001

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