

Biochemical Performance and Quantitative Assessment of F1 Hybrid of Two Ecoraces of Tropical Tasar Silkworm *Antheraea Mylitta* Drury (Lepidoptera: Saturniidae)

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Abstract

Antheraea mylitta Drury is basically a crossbreeding species, as such it seems to be potentially a good material for the exploitation of heterosis. In the present study F1 hybrid of wild ecorace Laria (L) and semi-domestic Daba (D) was raised and evaluated for various quantitative traits and biochemical parameters during larval stage. Improved fecundity ($+18 \pm 1.8\%$) and higher egg hatching rate ($+10.96 \pm 1.3\%$) was recorded in the F1 hybrid (L x D). Biochemical parameters studied in the hemolymph, midgut and fatbody of the larva showed significantly higher ($P < 0.05$) total proteins and carbohydrate concentration besides digestive enzyme activity. Correspondingly SDS-PAGE revealed more number of protein bands in the hemolymph sample of F1s, ranging between 29 kDa to 66 kDa compared to parental lines. The present study demonstrates the positive heterosis effect in the F1 hybrid of Laria x Daba. Biochemical analysis indicates that, there is possibilities of exploitation of hybrids with specific parents targeted for desirable commercial traits (silk yield and fecundity). Moreover, most of these biochemical parameters can be used as markers to analyze the genetic improvement in the tasar silkworms.

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Introduction

Tropical tasar culture involves rearing of tropical tasar silkworm *Antheraea mylitta* Drury in the forest or economic plantation of tasar food plants to produce tasar cocoons which further processed to extract world famous *vanya* silk. Presently, the annual contribution of tasar raw silk is about 1590 MT (2011-12) which has substantial demand in

the international market. Tasar silkworm is a polyphagous insect and differs in its adaptation on different food plants (Suryanarayana *et al.*, 2005). The tropical tasar silkworm populations occupying in different ecological and geographical regions show extensive phenotypic variability (Jolly *et al.*, 1968; Sengupta *et al.*, 1993; Singh and Srivastava, 1997; Srivastava *et al.*, 2003; Suryanarana and Srivastava, 2005) for which they are known as 'ecoraces'. About 44 ecoraces

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have been identified throughout India. Tasar silkworm is basically a cross breeding species, as such it seems to be potentially a good material for the exploitation of heterosis (Sengupta *et al.*, 1987). Since, variability being the genetic basis of any crops this is the basic requirement for genetic improvement. Therefore, in order to create new reservoirs of genetic variability, the use of crossing method the most genetically diverse parents has become essential as it helps in recombination of genes from diverse sources. The existing genetic variation among wild and domesticated sericigenous insects needs exploitation through hybridization for better quantitative and qualitative traits (Reddy *et al.*, 2008). To develop productive hybrids and high yielding segregates in *A. mylitta*, the pre-requisites are selection of genetically divergent parental ecoraces, identification of commercial traits with heterosis potential (Reddy *et al.*, 2010; Sengupta *et al.*, 1987; Siddiqui, 1997). The present study was to explore the potentialities of F1 hybrid produced from a wild ecorace Laria crossed with semi-domestic Daba ecorace of *A. mylitta*.

Material & Methods

Experiments of present work were undertaken at Silkworm Breeding and Genetics laboratories and rearing of silkworms at the farm of Central Tasar Research & Training Institute, Ranchi during the second crop rearing season (September-November, 2012).

Grainage and raising of F1 hybrid

Grainage was conducted during September 2012, Daba cocoons harvested during the first crop (July-August 2012) were collected from the Germplasm bank, Silkworm breeding section, Central Tasar Research & Training Institute. Laria cocoons were collected from forest area of Peterbar, Jharkhand. The good cocoons were sorted based on the sex. A total of 100 male and identical numbers of female cocoons each of two ecoraces were selected and stored at normal grainage conditions until the emergence of moth. When emergence started, crossings were made as Laria x Daba, besides the parental stocks. Mated female moths of all the batches were kept for oviposition for 3 days at normal grainage conditions.

Fecundity and egg hatching

The fecundity was calculated as total number of eggs laid by a single mother moth in three days of egg laying (Sinha, 1998). Hatching rate was recorded (Saheb *et al.*, 2009) and expressed in percentage.

Preparation of samples for biochemical studies

Hemolymph, fat body and midgut of Daba, F1 hybrid and Laria of both the sexes were collected separately during 5th day of V instar for the biochemical studies which is known to be the most active stage of tasar silkworm in terms of metabolism. Hemolymph and fat body samples were collected by dissecting out the larva and further processed (Lokesh *et al.*, 2012b). Midgut was collected by separating anterior and posterior part of the gut and transferred to a pre-cooled plastic vials and stored at -20°C until further use. Subsequently the tissues were homogenized with appropriate buffer, diluted and the supernatants were used for biochemical analysis.

Biochemical analysis

The total protein concentration was assayed as described by Lowry *et al.*, (1951), total carbohydrates was determined according to Sinha *et al.*, (1998). The digestive enzymes viz., amylase activity and protease activity were analyzed in the larval midgut and hemolymph. Soluble starch was used as a substrate to analyze the amylase activity as mentioned by Ishaaya and Swirsky (1976). Protease was assayed according to Eguchi and Iwamoto (1982). Acid phosphatase activity was recorded in the hemolymph and midgut as mentioned earlier by Gaikwad *et al* (2010). Alkaline phosphatase was analyzed in the larval midgut by the method described by Wilson and Walker (2000). The qualitative analysis of total soluble protein was done in haemolymph by using the SDS-PAGE as described by Zingales (1984).

Statistical analysis

The data generated in the present study is the average values of three replicates (n = 5 for biochemical analysis and n = 100 for quantitative trait analysis). One-way ANOVA was carried out using SPSS 10.0 statistical package to test the significance

Table 1. Fecundity and egg hatching of Daba, Laria and F1 hybrid (Mean \pm SE)

	Fecundity (nos.)	Egg hatching (%)
Daba	225 \pm 6.80	83 \pm 2.64
Laria	201 \pm 5.13	72 \pm 3.05
F ₁ hybrid	253 \pm 9.84	86 \pm 2.08

of differences, between the means of individuals and among the groups studied. Comparisons were performed with Duncan's Multiple Range Test (DMRT, $P < 0.05$) (Duncan, 1955).

Results

The performance of the F1 hybrid (Laria x Daba) tasar silkworm was analyzed and compared with its parental lines for fecundity, egg hatching and biochemical parameters are recorded.

Fecundity and Egg hatching

The performance of F1 hybrid was recorded with significant improvement in the fecundity ($P < 0.05$) over its parental lines (Table 1). The fecundity was 253 ± 6.51 in F1 hybrid compare to the parental lines, Daba 225 ± 5.74 and Laria 201 ± 7.91 there was a genetic gain of about 18% over the mid parent value for the fecundity. Higher hatching was recorded in the F1 hybrids (86%) compared to the parental lines, Daba 83% and Laria recorded 72% of hatching in three days.

Total protein concentration

Protein concentration was recorded highly variable between the different batches (Daba, Laria and F1 hybrid) and the tissues studied. Higher protein concentration was recorded in the hemolymph of F1 hybrid female larvae (372.00 ± 1.73 mg/ml) followed by Daba female larval hemolymph (368.00 ± 1.15 mg/ml) and the lowest concentration (106.00 ± 1.73 mg/ml) was recorded in the fat body of male Laria larva. A significant increase in the protein concentration ($P < 0.05$) in the F1 hybrid was recorded, the values obtained is higher than either of the parents. Also, variability

in the protein concentration among the tissues studied, higher proteins was observed in the hemolymph followed by midgut tissue and fat body (Table 2). Sexual dimorphism was very much apparent, where higher protein concentration was observed in females of all the batches when compared to males.

Total Carbohydrates

The level of carbohydrates was recorded the significantly higher ($P < 0.05$) in the F1 hybrid compared to the parental lines (Table 2). It was 42.13 ± 0.88 mg/ml and 37.30 ± 0.57 in the hemolymph, 32.40 ± 0.57 and 30.10 ± 0.1 in midgut tissue and 25.10 ± 1.15 and 23.90 ± 1.15 mg/ml in fat body of F1 hybrid. The lower values (22.10 ± 1.15 and 23.90 ± 1.15 mg/ml) for total carbohydrates were recorded in the fat body of Laria ecorace followed by fat body of Daba (21.96 ± 1.45 & 24.80 ± 2.30 mg/ml) and F1 hybrid (23.90 ± 1.15 & 25.10 ± 1.15 mg/ml).

Digestive enzymes

The level of activity showed significant variations among the larval batches viz., Daba, Laria and F1 hybrid. Significantly higher ($P < 0.05$) activity was found in the midgut tissue of F1 hybrid larvae (male 0.820 ± 0.011 and female 0.79 ± 0.017 μ M/ml of homogenate) compared to other samples. Similar observations were made in the level of the protease activity. Activity found minimum in Laria larval hemolymph. Higher activity was found in mid gut tissue of all the batches compared to enzyme activity in hemolymph (Table 3).

Acid and Alkaline phosphatase

An increase in the phosphatase activity was found in the midgut tissue of F1 hybrid larva the level of the activity was significantly higher to the parental lines (Table 4).

Electrophoresis of hemolymph protein

Qualitative analysis of haemolymph proteins in the V instar larva of Daba, Laria and F1 hybrid of tasar silkworm was recorded (Fig. 1) Polymorphic variations were noticed with regard to the number of protein bands in male and female also in different batches viz., Daba, Laria and F1 hybrid. Protein bands of 98 KDa, 70 kDa, 45

Table 2. Quantitative analysis of total protein and carbohydrate concentrations in the different tissues of Daba, Laria & F1 hybrid (Mean \pm SE)

	Protein concentration (mg/g tissue)						Total Carbohydrates (mg/g tissue)					
	Daba		Laria		F1 hybrid		Daba		Laria		F ₁ hybrid	
	M	F	M	F	M	F	M	F	M	F	M	F
hemo-lymph	308 ± 1.15	368 ± 1.15	293 ± 1.15	318 ± 0.57	355 ± 1.15	372** ± 1.73	33.13 ± 1.15	38.67 ± 1.27	30.10 ± 1.15	36.30 ± 1.73	37.30 ± 0.57	42.13** ± 0.88
fat body	114 ± 0.57	123 ± 1.15	106 ± 1.73	116 ± 1.15	123 ± 1.73	135 ± 1.15	21.97 ± 1.45	4.80 ± 2.30	22.10 ± 1.15	23.90 ± 1.15	23.90 ± 1.15	25.10** ± 1.15
midgut	190 ± 1.73	250 ± 2.88	178 ± 1.73	225 ± 2.88	222 ± 1.00	283** ± 1.15	28.10 ± 1.00	29.80 ± 1.15	27.30 ± 1.15	28.50 ± 0.57	30.10 ± 1.00	32.40** ± 0.57
F value: 3786.557 CD at 5%						F value: 25.725 CD at 5%						

** Significant at $p < 0.05$, M – Male, F – Female

Table 3. Activity analysis of digestive enzymes in the different tissues of Daba, Laria & F1 hybrid (Mean \pm SE)

	Amylase activity (μ mole maltose/min/mg protein)						Protease activity (μ mole tyrosine/min/mg protein)					
	Daba		Laria		F1 hybrid		Daba		Laria		F1 hybrid	
	M	F	M	F	M	F	M	F	M	F	M	F
hemo-lymph	0.51 ± 0.00	0.55 ± 0.01	0.47 ± 0.01	0.50 ± 0.11	0.55 ± 0.01	0.61 ± 0.00	0.75 ± 0.01	0.79 ± 0.01	0.70 ± 0.00	0.72 ± 0.01	0.79 ± 0.00	0.82 ± 0.01
midgut	0.69 ± 0.11	0.78 ± 0.00	0.62 ± 0.00	0.71 ± 0.01	0.82 ± 0.01	0.79** ± 0.01	0.90 ± 0.00	0.92 ± 0.00	0.85 ± 0.01	0.88 ± 0.00	0.95 ± 0.01	0.99** ± 0.00
F value: 6.415 CD at 5%						F value: 109.597 CD at 5%						

** Significant at $p < 0.05$, M – Male, F – Female

Table 4. Activity analysis of Acid and Alkaline Phosphatase in the different tissues of Daba, Laria & F1 hybrid (Mean \pm SE)

	Acid Phosphatase (μ g p-nitro phenol/mg protein)						Alkaline Phosphatase (midgut) (μ g p-nitro phenol/mg protein)					
	Daba		Laria		F1 hybrid		Daba		Laria		F1 hybrid	
	M	F	M	F	M	F	M	F	M	F	M	F
hemo-lymph	43.6 ± 0.57	47.1 ± 1.15	41.8 ± 1.15	44.5 ± 0.57	44.5 ± 0.01	47.8 ± 1.15	ND	ND	ND	ND	ND	ND
midgut	54.4 ± 0.57	58.1 ± 1.73	53.0 ± 1.15	55.9 ± 1.15	60.1 ± 0.57	66.3** ± 1.15	25.07 ± 0.57	27.8 ± 1.15	20.3 ± 1.15	24.3 ± 1.15	24.9 ± 1.15	27.2** ± 0.57
F value: 57.853 CD at 5%						F value: 29.975 CD at 5%						

** Significant at $p < 0.05$, M – Male, F – Female, ND – Not determined

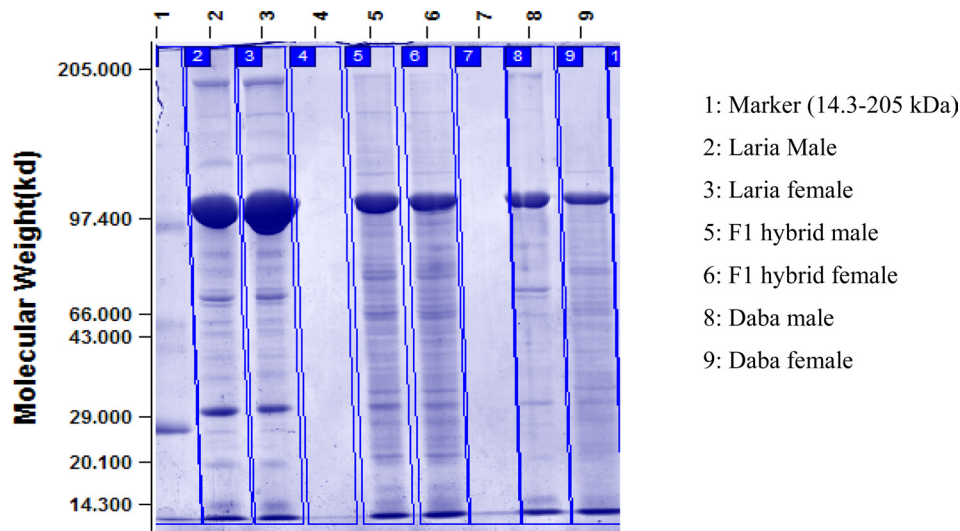


Fig. 1. Electrophoresis analysis of 5th Instar tasar silkworm larval hemolymph of Daba, Laria and F1 hybrid.

kDa, 28 kDa and 14 kDa are found common in all the hemolymph samples. Additional number of protein bands was recorded in the hemolymph sample of F1 hybrid ranging between 29 kDa to 66 kDa compared to the samples of Daba and Laria. Conspicuously stained bands of 67 kDa appeared in the hemolymph samples of F1 hybrid.

Discussion

The quantitative characters in silkworm are highly variable and have a greater economic value. F1 hybrids are most commonly used for commercial cocoon production because they represent high heterosis for most of the economic characters (Singh *et al.*, 2012). Earlier, it has been reported that F1 hybrids are less variable than parental lines, three-way and double crosses. The heterosis in silkworm, many characters have been found to have a relationship with the qualitative and quantitative aspects of silk yield (Ohio *et al.*, 1970). The improvement observed in some of the quantitative traits of the F1 hybrid in relation to fecundity and egg hatching/fertility in present study shows a positive heterosis effect as a result of genetically divergent crosses. Fecundity is considered as one of the most desired quantitative traits of commercial importance in silkworms (Reddy *et al.*, 2010). The genotype-environment interaction has highly significant influence on the fecundity of the silkworms, the differential performance between the groups viz., F1 hybrid, Daba and Laria with reference to fecundity attributed to the genetic endowment of the two ecoraces (Omana and Gopinathan, 1995). Similarly

the egg hatching is depends on the fertilization of eggs and proper development of embryo inside the egg (Saheb *et al.*, 2009). The higher fecundity and hatching rate might be due to the epistasis effect as a result of crossing two different tasar silkworm ecoraces (Falconer, 1985; Aruga, 1994; Reddy *et al.*, 2010) also this can be attributed that positive gain in the silkworm traits might be due to expression of dominant gene(s) for a character (Verma *et al.*, 2005; Reddy *et al.*, 2008).

Higher concentration of the total protein and carbohydrates recorded in the present study for F1 hybrid is attributed to the fact that these larvae have acquired robustness due to the inter-ecoracial crossing and fed higher quantity and quality leaves in turn efficiently converted the dietary food during its feeding stage, the corresponding level of proteins were maintained in the hemolymph, midgut and fat body. Also, many authors have similarly recorded higher proteins in the hemolymph of female earlier (Babu *et al.*, 2009; Kumar *et al.*, 1998; Lokesh *et al.*, 2012a; Kumar *et al.*, 2011; Srivastava *et al.*, 2001). The differential concentration recorded in different batches viz., Daba Laria and F1 hybrid depicts the differential feeding rate and physiological activity among different ecoraces and adaptively for the geographical region and food plant (Venugopala Pillai *et al.*, 1987). The digestive enzymes viz., amylase and protease in both midgut and haemolymph of tropical tasar silkworms revealed that the activity was higher in the midgut compared to hemolymph. This is mainly due to the fact that, the maximum leaves consumed by the larvae were during that period and the presence of food in the gut may act as a stimulus for the

secretion of enzymes similar to the findings of Lokesh and Ananthanarayana (2012) observed in the silkworm *Bombyx mori*. Ishaaya *et al.* (1971) showed in the larvae of *Spodoptera littoralis* that the protein factors act as stimulation for digestive enzymes probably through a hormonal mechanism. Higher quantity of food accumulated in the digestive tract, leads for more secretion of digestive enzymes in the midgut (Waldbauer, 1968). Acid and Alkaline phosphatases are hydrolases, is a group of enzymes that catalyzes the hydrolysis of a chemical bond during a metabolic process and is mainly involved in lysosomal activities. The significant increase in the levels of key hydrolases such as acid and alkaline phosphatases in silk gland and fat body may be due to the rupturing of various cellular and lysosomal membranes that contain the hydrolytic enzymes, resulting in an increase in their activities (Singh *et al.*, 2010). The higher acid and alkaline phosphatase activity recorded in the mid gut of F1 hybrid is in corresponding with the level of other digestive enzyme activity. Where, phosphatases hydrolyse variety of phosphate monoesters of consumed leaves in the larval gut and transphosphorylated into silkworm biomolecules. The level of phosphatase activity in the mid gut is correlated with that of silk protein synthesis level and absorption capacity of digested food in the silkworm larvae (Gaikwad *et al.*, 2010).

Study of protein banding patterns by SDS-PAGE in the hemolymph samples drawn from different larval batches revealed that the number of protein bands and specificity of bands vary between the larval batches and also between male and female. The presence and absence of few protein bands at different molecular weight indicates the synthesis of a particular protein to meet the physiological need. Protein bands of 98 kDa, 70 kDa, 45 kDa, 28kDa and 14 kDa are found common in all the hemolymph samples of all the larva batches. This could be of species specific proteins, presumed to found in all the ecoraces of the *A. mylitta*. Kumar *et al.*, (2011) showed the protein bands of 36 and 64 kDa are constitutively appeared in all the tissues studies in *A. mylitta*. More number of protein bands was observed in the hemolymph sample of F1 hybrid between 66 kDa to 29 kDa compared to the samples of Daba and Laria. This is attributed to the higher protein concentration recorded in the hemolymph of F1 hybrid. Conspicuous bands of 67 kDa are appeared in the hemolymph samples of F1 hybrid can be interpreted that higher synthesis of this protein. The higher number of protein bands in the F1 hybrid indicated the improvement of protein synthesis and concentration

over its parental lines i.e., Daba and Laria.

Better performance of F hybrid of Laria x Daba in almost all the parameters studied in the present study indicates the possible influence of hybridization. Also a positive heterosis effect in the F1 hybrid this envisages the possible utilization of F1 hybrids from the crosses of wild and semi-domesticated ecoraces of tropical tasar silkworm for optimal silk productivity. However, biochemical analysis envisages that, there will be possibilities of breeding with specific parents obtaining desirable commercial traits (silk yield and fecundity) are an impending tool to optimize heterosis for genetic improvement in the desired direction. Moreover, these biochemical parameters can be used as markers to analyze the genetic improvement in the tasar silkworms especially in hybrids.

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