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Biochemical Performance and Quantitative Assessment of F1 Hybrid of Two Ecoraces of Tropical Tasar Silkworm Antheraea Mylitta Drury

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Abstract: Antheraea mylitta drury is essentially a crossbreeding species, thus it appears to be a potentially good material for exploiting heterosis. In the present study, F1 hybrids of wild Eochres larrea (L) and semi-domesticated daba (D) were raised and evaluated for various quantitative traits and biochemical parameters during the larval stage. Improved fertility (+18 ± 1.8%) and higher egg hatching rate (+10.96 ± 1.3%) were recorded in the F1 hybrids (L x D). The biochemical parameters studied in hemolymph, midgut and fatbody of the larvae showed significantly increased ($P<0.05$) total protein and carbohydrate concentrations besides digestive enzyme activity. Similarly SDS-PAGE revealed higher number of protein bands in the hemolymph sample of F1, ranging from 29 kDa to 66 kDa, compared to the parental lines. The present study demonstrates positive heterosis effect in F1 hybrid of Laria x Daba. Biochemical analysis indicates that there is potential for exploiting hybrids with specific parents targeted for desirable commercial traits (silk yield and fertility). Moreover, most of these biochemical parameters can be used as markers to analyse genetic improvement in tasar silkworms.

Keywords: F1 hybrid, *Antheraea mylitta*, Proteins, Daba, Laria, Ecoraces etc.

1. Introduction

Tropical tasar culture involves rearing of the tropical tasar silkworm Antheraea mylitta drury in the wild or economic plantation of tasar food plants to produce tasar cocoons which are further processed to extract the world famous Vanya silk. At present, the annual contribution of tasar raw silk is about 1590 metric tons (2011-12) which has considerable demand in the international market. Tasar silkworm is a polyphagous insect and varies in its adaptations to different food plants (Suryanarayana et al., 2005). Populations of tropical tasar silkworm living in different ecological and geographical regions show wide phenotypic variability (Jolly et al., 1968; Sengupta et al., 1993; Singh and Srivastava, 1997; Srivastava et al., 2003; Suryanarayana and Srivastava, 2005) for which they are known as 'ecores'. About 44 ecoraces have been identified all over India. Tasar silkworm is essentially a cross breeding species, thus it appears to be a potentially good material for exploiting heterosis (Sengupta et al., 1987). Since variability is the genetic basis of any crop, it is the basic requirement for genetic improvement. Therefore, to create new reservoirs of genetic variability, use of crossing method with most genetically diverse parents has become essential as it helps in recombination of genes from different sources. The existing genetic variation among wild and domesticated sericigenous insects needs to be exploited through hybridization for improved quantitative and qualitative traits (Reddy et al., 2008). To develop productive hybrids and high yielding isolates in A. mylitta, the pre-requisites are selection of genetically different parental ecotypes, identification of commercial traits having heterosis potential (Reddy et al., 2010; Sengupta et al., 1987; Siddiqui, 1997). The aim of the present study was to explore the potential of F1 hybrids produced from the wild ecotype Larrea crossed with the semi-domesticated Daba ecotype of A. mylitta.

2. Materials & Method

The experiments of the present work were carried out in the silkworm breeding and genetics laboratories and in the farm of Central Tasar Research and Training Institute, Ranchi during the second rearing season.

Grainage and raising of F1 hybrid

Grain collection was done during September 2012, Daba cocoons harvested during first harvest (July-August 2012) were collected from Germplasm Bank, Silkworm Breeding Section, Central Tasar Research & Training Institute. Laria cocoons were collected from forest area of Peterbar, Jharkhand. Good cocoons were sorted on the basis of sex. A total of 100 male and equal number of female cocoons of two ecotypes were selected and stored under normal grain conditions till the emergence of moths. When emergence started, crossing was done as Laria x Daba apart from parental stock. The mated female moths of all batches were kept under normal grain conditions for 3 days for egg formation.

Fecundity and egg hatching

Fecundity was calculated as the 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

For biochemical studies, the hemolymph, fat body and midgut of Daba, F1 hybrid and Larrea of both sexes were collected separately on the 5th day of V instar, which is considered to be the most active stage of tasar silkworm in terms of metabolism. Hemolymph and fat body samples were collected by dissecting the larvae and further processed (Lokesh et al., 2012b). The midgut was collected by separating the anterior and posterior part of the intestine and transferred to pre-chilled plastic vials and stored at -20°C until further use. The tissues were then homogenized with appropriate buffers, diluted and the supernatant was used for biochemical analysis.

Biochemical analysis

Total protein concentration was tested as described by Lowry et al., (1951), total carbohydrate was determined as per Sinha et al., (1998). Digestive enzymes viz. amylase activity and protease activity were analyzed in larval midgut and hemolymph. Soluble starch was used as substrate for analyzing amylase activity as mentioned by Ishaya and Swirsky (1976). Protease was tested as per Eguchi and Iwamoto (1982). Acid phosphatase activity was recorded in hemolymph and midgut as mentioned earlier by Gaikwad et al. (2010). Alkaline phosphatase was analyzed in larval midgut by the method described by Wilson and Walker (2000). Qualitative analysis of total soluble protein was done in hemolymph using SDS-PAGE as described by Zingales (1984).

Statistical analysis

The data generated in the present study are the average values of three replicates (n = 5 for biochemical analysis and n = 100 for quantitative trait analysis). One-way ANOVA was performed using the SPSS 10.0 statistical package to test the significance of differences between the means of individuals and the studied groups. Comparisons were made with Duncan's multiple range test (DMRT, $P<0.05$) (Duncan, 1955).

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

	Fecundity (nos.)	Egg hatching (%)
Daba	225 ± 6.80	83 ± 2.64
Laria	201 ± 5.13	72 ± 3.05
F_1 hybrid	253 ± 9.84	86 ± 2.08

3. Results

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

Fecundity and Egg hatching

The performance of F1 hybrids was recorded with significant improvement in fertility ($P<0.05$) as compared to its parental lines (Table 1). Fertility in F1 hybrids as compared to parental lines was 253±6.51, with Daba recording 225±5.74 and Laria recording 201±7.91 as compared to the median parental value for fertility, a genetic gain of about 18%. High hatching (86%) was recorded in F1 hybrids as compared to parental lines, with Daba recording 83% and Laria recording 72% hatching within three days.

Total protein concentration

Protein concentrations were recorded to be highly variable among different batches (Daba, Laria and F1 hybrid) and tissues studied. Highest protein concentration (372.00±1.73 mg/ml) was recorded in the hemolymph of F1 hybrid female larvae, followed by Daba female larvae 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 larvae. A significant increase in protein concentration ($P<0.05$) was recorded in the F1 hybrid, the value obtained being higher than that of either of the parents. Further, variability in protein concentrations was observed in the tissues

studied, higher protein was observed in hemolymph, followed by midgut tissue and fat body (Table 2). Sexual dimorphism was very evident, where higher protein concentrations were observed in females of all batches compared to males.

Total Carbohydrates

The carbohydrate level was recorded to be significantly higher (P<0.05) in F1 hybrids than in the parental lines (Table 2). It was 42.13±0.88 mg/ml and 37.30±0.57 in hemolymph, 32.40±0.57 and 30.10±01 in midgut tissue and 25.10±1.15 and 23.90±1.15 mg/ml in fat body of F1 hybrids. The lowest values for total carbohydrates (22.10±1.15 and 23.90±1.15 mg/ml) were recorded in the fat body of Larrea echres, followed by Daba (21.96±1.45 and 24.80±2.30 mg/ml) and F1 hybrid (23.90±1.15 and 25.10±1.15 mg/ml).

Digestive enzymes

Significant variations were observed in the activity levels among larval batches viz., Daba, Larrea and F1 hybrid. Significantly higher (P<0.05) activity was found in the mid gut tissue of F1 hybrid larvae (male 0.820±0.011 and female 0.79±0.017 µM/ml homogenate) compared to other samples. Similar observations were also made in the protease activity levels. The activity was found to be lowest in Larrea larval hemolymph. Higher activity was found in mid gut tissue of all batches compared to the enzyme activity in hemolymph (Table 3).

Acid and Alkaline phosphatase

Increased phosphatase activity was found in the mid-intestinal tissue of F1 hybrid larvae, with activity levels being significantly higher than those of the parental lines (Table 4).

Electrophoresis of hemolymph protein

Qualitative analysis of haemolymph proteins in V instar larvae of Daba, Laria and F1 hybrid of tasar silkworm was recorded (Fig. 1). Polymorphic variations were observed with respect to number of protein bands in male and female in different batches viz. Daba, Laria and F1 hybrid. Protein bands of 98 KDa, 70 kDa, 45 kDa, 28 kDa and 14 kDa were found common in all haemolymph samples. Excess number of protein bands ranging between 29 kDa to 66 kDa were recorded in haemolymph sample of F1 hybrid as compared to Daba and Laria samples. Clearly stained band of 67 kDa was seen in haemolymph samples of F1 hybrid.

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

	Protein concentration (mg/g tissue)						Total Carbohydrates (mg/g tissue)					
	Daba		Laria		F1 hybrid		Daba		Laria		F1 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%					

Table 3. Activity analysis of digestive enzymes in the different tissues of Daba, Laria & F1 hybrid (Mean ±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 ±.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%					

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

	Acid Phosphatase (µg p-nitro phenol/mg protein)						Alkaline Phosphatase (midgut) (µg 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%					

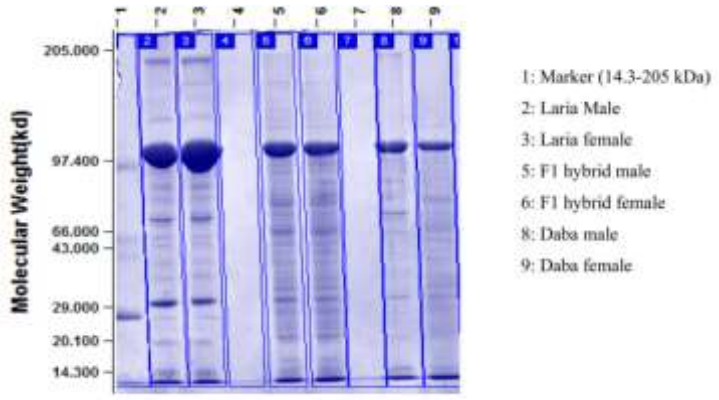

Figure 1. Electrophoresis analysis of 5th Instar tasar silkworm larval hemolymph of Daba, Laria and F1 hybrid.

4. Discussion

Quantitative traits in silkworm are highly variable and have high economic value. F1 hybrids are commonly used for commercial cocoon production as they represent high heterosis for most of the economic traits (Singh et al, 2012). Previously, it has been reported that F1 hybrids are less variable than parental lines, three-way and double crosses. Heteroscedasticity in silkworm, several traits have been found to be correlated with qualitative and quantitative aspects of silk yield (Ohio et al, 1970). The improvement observed in some quantitative traits of F1 hybrids with respect to fertility and egg hatching/fertility in the present study shows positive heterosis effect as a result of genetically different crosses. Fertility is considered to be one of the most desired quantitative traits of commercial importance in silkworm (Reddy et al, 2010). Genotype-environment interaction has a very significant impact on the fertility of silkworms, with differential performance among groups such as F1 hybrids, daba and lariah in terms of fertility due to genetic potential of two ecotypes (Omana and Gopinathan, 1995). Similarly, hatching of eggs depends on fertilization of eggs and proper development of embryo inside the egg (Sahab et al., 2009). High fertility and hatching rate may be due to epistasis effect as a result of crossing of two different tasar silkworm ecotypes (Falconer, 1985; Aruga, 1994; Reddy et al., 2010), also it can be assumed that positive gains in silkworm traits may be due to expression of dominant gene(s) for a character (Varma et al., 2005; Reddy et al., 2008). The higher concentration of total protein and carbohydrates recorded in the present study for F1 hybrids is due to the fact that these larvae have gained robustness due to inter ecological crossing and fed on higher quantity and quality leaves, in turn efficiently converted dietary food during their feeding stage, corresponding levels of protein were maintained in hemolymph, midgut and fat body. Moreover, several authors have previously recorded higher protein in hemolymph of females (Babu et al., 2009; Kumar et al., 1998; Lokesh et al., 2012a; Kumar et al., 2011; Srivastava et al., 2001). The differential concentrations recorded in different batches viz., Daba laria and F1 hybrids reflect differential feeding rate and physiological activity among different ecological species and also reflect adaptation to geographical region and food plant (Venugopal Pillai et al., 1987). Digestive enzymes viz., amylase and protease in both midgut and haemolymph of tropical tasar silkworm revealed that the activity was higher in midgut than in haemolymph. This is mainly due to the fact that, maximum leaves eaten by the larvae were during that period and the presence of food in the gut may act as a stimulus for secretion of enzymes, as observed by Lokesh and Ananthanarayan (2012) in silkworm Bombyx mori. Ishaya et al. (1971) showed in larvae of Spodoptera littoralis that protein factors act as stimuli for digestive enzymes, possibly through a hormonal mechanism. Greater quantity of food deposited in the digestive tract leads to greater secretion of digestive enzymes in the midgut (Waldbauer, 1968). Acid and alkaline phosphatases are hydrolases, a group of enzymes that catalyze the hydrolysis of a chemical bond during a metabolic process and are mainly involved in lysosomal activities. The significant increase in the levels of major hydrolases such as acid and alkaline phosphatase in the silk gland and fat body may be due to the breakdown of various cellular and lysosomal membranes containing hydrolytic enzymes, resulting in increased their activities (Singh et al., 2010). The high acid and alkaline phosphatase activity recorded in the midgut of F1 hybrids is consistent with the levels of other digestive enzyme activity. Where, phosphatases hydrolyze variety of phosphate monoesters of consumed leaves in the larval gut and transphosphorylate them into silkworm biomolecules. The level of phosphatase activity in the midgut is correlated with silk protein synthesis level and absorption capacity of digested food in silkworm larvae (Gaikwad et al., 2010).

The study of protein banding pattern by SDS-PAGE in haemolymph samples taken from different larval batches revealed that the number of protein bands and the specificity of the bands vary among larval batches and also between male and female.

The presence and absence of certain protein bands at different molecular weights indicates synthesis of a particular protein to meet the physiological requirement. Protein bands of 98 kDa, 70 kDa, 45 kDa, 28 kDa and 14 kDa are found common in all haemolymph samples of all larval batches. These may be species-specific proteins, found in all ecotypes of A. mylitta. Kumar et al., (2011) showed that protein bands of 36 and 64 kDa appear constitutively in all tissue studies in A. mylitta. A higher number of protein bands between 66 kDa to 29 kDa were observed in haemolymph sample of F1 hybrids as compared to samples from Daba and Laria. This is because of the higher protein concentration recorded in the hemolymph of F1 hybrids. The hemolymph samples of F1 hybrids showed specific bands of 67 kDa, which can be interpreted to mean higher synthesis of this protein. The higher number of protein bands in F1 hybrids indicated improved protein synthesis and concentration compared to its parental lines i.e. Daba and Laria. The better performance of F hybrid of Laria x Daba in almost all parameters studied in the present study indicates the potential effect of hybridization. There is also positive heterosis effect in F1 hybrids, which envisages the potential use of F1 hybrids from the cross of wild and semi-domesticated ecotypes of tropical tasar silkworm for optimum silk productivity. However, biochemical analysis envisages that, there will be possibilities of breeding with specific parents achieving the desired commercial traits (silk yield and fertility), which is an imminent tool to optimize heterosis for genetic improvement in the desired direction. Moreover, these biochemical parameters can be used as markers to analyze genetic improvement in tasar silkworms especially in hybrids.

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