



## Differential Digestive Amylase Activity in Silkworm, *Bombyx mori* L. in Relation with Biological and Yield Traits

K.A. Murugesha\*, S. Mohankumar and C.A. Mahalingam

\*Department of Sericulture and

<sup>1</sup>Department of Plant Molecular Biology and Biotechnology  
Tamil Nadu Agricultural University, Coimbatore -641 003

A study was undertaken to ascertain the variability in digestive amylase activity in different mulberry silkworm races. PA12 recorded the highest larval and cocoon weight (1.52 g and 0.68 g) in a pool of multivoltines tested and CSR2 recorded the highest (3.08 g and 2.49 g) among bivoltines tested. Among multivoltines, Nistari (1722.5 µg) and CB5 (1648.8 µg) recorded higher amylase activity and Tamil Nadu White recorded the least. Among bivoltines, the amylase activity was significantly higher in PAM101 (184.03µg) and NB4D2 (180.42µg). CSR19, CC1, NB7 and CSR18 were on par and showed low digestive amylase activity. Amylase activity in digestive juice was about 10 to 15 fold higher in multivoltines than bivoltines. Positive significant correlation ( $P=0.01$ ) was recorded between digestive amylase activity and effective rate of rearing (0.812). Negative correlation was recorded between amylase activity, and biological and yield related traits of silkworm, *Bombyx mori* L.

**Keywords:** Digestive amylase, *Bombyx mori*, multivoltine, bivoltine, ERR.

The digestibility in silkworm, *Bombyx mori* L. largely depends upon the activity of the enzyme amylase. Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects (Horie and Watanabe, 1983) particularly carbohydrates available in the form of starch in mulberry leaves (Chatterjee *et al.*, 1989). The ability to digest more food may influence the growth, development, resistance to diseases and stress and better survival under different environmental conditions. Screening of large number of silkworm stocks of wide geographical origin and yield status at different research centers have clearly indicated wide genetic variability in the activity of digestive amylase (Hirata and Gamo, 1969; Chatterjee *et al.*, 1992). Hirata (1971) reported the relationship between digestive amylase activity and economic traits among silkworm races. Most of the high yielding, diapausing stocks had low amylase activity while most of the low yielding non-diapausing stocks showed high amylase activity. In general, the activity of amylase in multivoltine races was almost two fold higher than the bivoltine races of silkworms (Chatterjee *et al.*, 1992). The productivity was comparatively less and survival rate was higher in larvae with high amylase activity but in strains with low amylase activity the reverse was noticed. Crossing both of them will result in getting the advantages of productivity and effective rate of rearing. Hence, the investigations were carried out to study the differential digestive amylase activity in

mulberry silkworm in relation with biological and yield related parameters.

### Materials and Methods

The investigations were conducted at the laboratories of Department of Sericulture, Centre for Plant Protection Studies and Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. Disease free layings of different races of *B. mori* L. were obtained from Central Sericultural Germplasm Resources Centre (CSGRC), Hosur and Central Sericultural Research and Training Institute, Mysore. Rearing of silkworms was carried out utilizing the irrigated mulberry (cv.V-1) by adopting the practices suggested by Krishnaswami (1978).

#### Experimental set-up

Experimental design	: Completely Randomized Design (CRD)
No. of treatments	: 30
No. of replications/ treatment	: 3
No. of larvae/ replication	: 50

#### Estimation of digestive amylase activity

Four day old fifth instar worms of each of 30 races were collected randomly from the rearing bed. They were starved for 2 h and kept in a clean, closed container containing dried tobacco leaves. The

\*Corresponding author email: murugeska2002@yahoo.co.in

starved larvae were placed on plastic paper over the leaves. After 20 minutes, the regurgitated digestive juice on the plastic paper was collected with syringes and samples were stored at -20°C to avoid oxidation. They were centrifuged at 5000 rpm for 5 minutes in 4°C to get clear solution, that were used for further analysis. Quantification was done as per the standard procedure (Manickam and Sadasivam, 1992). The digestive amylase activity was recorded by amount of maltose released from starch in one hour reaction and correlated with biological and yield related parameters. The biological traits *viz.*, egg hatching (%), larval weight (g), cocoon weight (g), pupal weight (g) and shell weight (g), effective rate of rearing (ERR - %) and economic traits *viz.*, single filament length (m) and denier were recorded.

## Results and Discussion

### Biological traits

#### Larval weight

The larval weight was higher in bivoltine races as compared to multivoltines. Among the multivoltine, the significantly higher larval weight was

recorded in PA12 (1.524 g) and the least was recorded by C.Nichi (0.842 g). Similar types of results were reported by scientists from CSR&TI, Mysore (Anonymous, 1982) and Kalpana *et al.* (1994). In bivoltines, CSR2 recorded 3.088 g of larval weight. The larval weight in CSR races was not significantly different. Other than CSR races, NB4D2 ranked first with larval weight of 2.489 g followed by SH6 (2.130 g). The lowest larval weight was recorded in NB7 (1.826 g) followed by CA2 (1.867 g) and CC1 (1.936 g) (Table 1 & 2). Basavaraja (2001) also reported similar results.

#### Effective Rate of Rearing (ERR)

The effective rate of rearing ranged from 72.6 to 94.98 per cent in multivoltines. The race, Pure Mysore recorded statistically superior ERR (94.98 %) than all other races tested. In bivoltines, the effective rate of rearing ranged from 63.53 to 73.59 per cent. The significant variations observed in the phenotypic manifestations of both multivoltine and bivoltines for the characters analysed can be attributed to the genetic endowments and their degree of response to the environmental conditions to which they were exposed during growth and

**Table 1. Biological and yield related parameters of multivoltine silkworm races\***

Race	Biological parameter			Yield related parameter		
	Larval weight (g)	ERR (%)	Cocoon weight (g)	Shell weight (g)	Single filament length (m)	Denier
Kolar Gold	1.181 ± 0.03	87.09 ± 1.1	0.669 ± 0.023	0.098 ± 0.005	641.72 ± 7.4	2.11 ± 0.4
Kollegal Jawan	1.190 ± 0.05	90.82 ± 0.9	0.595 ± 0.024	0.094 ± 0.006	696.73 ± 5.6	1.54 ± 0.3
C. Nichi	0.842 ± 0.02	88.39 ± 0.5	0.558 ± 0.023	0.063 ± 0.002	397.32 ± 2.8	1.58 ± 0.3
Sarupat	0.914 ± 0.07	84.08 ± 1.3	0.512 ± 0.019	0.063 ± 0.003	354.05 ± 6.7	2.82 ± 0.5
P4D3	1.295 ± 0.04	85.25 ± 1.0	0.545 ± 0.032	0.066 ± 0.003	350.12 ± 3.7	2.06 ± 0.4
PA12	1.524 ± 0.08	82.53 ± 2.3	0.683 ± 0.017	0.105 ± 0.007	510.99 ± 7.8	2.52 ± 0.4
Tamil Nadu white	1.432 ± 0.04	78.12 ± 0.9	0.674 ± 0.019	0.107 ± 0.003	421.68 ± 6.3	2.24 ± 0.4
Hosa Mysore	1.341 ± 0.05	72.60 ± 0.4	0.671 ± 0.028	0.098 ± 0.003	332.07 ± 8.6	2.15 ± 0.4
Mysore Princess	1.099 ± 0.02	82.98 ± 1.9	0.522 ± 0.021	0.082 ± 0.005	324.83 ± 5.6	1.83 ± 0.3
CB5	0.954 ± 0.04	75.67 ± 1.8	0.519 ± 0.012	0.092 ± 0.005	331.29 ± 5.8	1.83 ± 0.3
Nistari	1.456 ± 0.04	88.94 ± 0.9	0.494 ± 0.007	0.110 ± 0.003	382.43 ± 6.3	2.29 ± 0.4
Pure Mysore	0.941 ± 0.03	94.98 ± 0.9	0.526 ± 0.023	0.083 ± 0.003	306.58 ± 4.3	1.04 ± 0.2
SEd	0.0646	1.877	0.0306	0.0059	8.607	0.0618
CD (0.05%)	0.1281	3.873	0.0607	0.0117	17.4346	0.1242

\*Mean of ten replications with Mean ± SD

development (Table 1 & 2). These observations are in line with the findings of Murugesh *et al.* (2007), who also observed maximum ERR in multivoltine races.

#### Yield related traits

##### Cocoon weight

Cocoon weight of multivoltine races ranged from 0.494 g to 0.683 g. The lowest cocoon weight was recorded by Nistari (0.494 g). In bivoltines, CSR2 recorded the significantly higher cocoon weight as 2.491 g. Similar type of observations were recorded for cocoon weight in bivoltine races as compared to

multivoltines (Table 1 & 2). These results are to the findings of Thangavelu (1997) and Basavaraja (2001), who recorded the maximum cocoon weight of 1.7 to 1.9 g in CSR races.

##### Shell weight

Thangavelu (1997) reported the variation in yield related parameters in multivoltines. Significantly higher shell weight was recorded in Nistari race followed Tamil Nadu White and PA12. Bivoltine races exhibited more than two fold increase shell weight than multivoltine races. CSR2 recorded the statistically superior shell weight of 0.556 g than

**Table 2. Biological and yield related parameters of bivoltine silkworm races\***

Race	Biological parameter			Yield related parameter		
	Larval weight (g)	ERR (%)	Cocoon weight (g)	Shell weight (g)	Single filament length (m)	Denier
NB4D2	2.489±0.01	73.59±0.77	1.782± 0.008	0.349±0.03	796.57 ±3.41	2.5±0.05
SH6	2.130±0.06	70.27±0.94	1.411 ±0.010	0.257±0.02	692.39 ±3.18	1.8±0.02
YS3	2.094±0.05	67.90±0.52	1.415 ±0.005	0.261±0.02	773.10 ±4.85	1.6±0.03
CSR2	3.088±0.09	72.39±0.81	2.491 ±0.004	0.556±0.05	1165.02 ±6.5	3.13±0.03
CSR3	2.819±0.02	64.63±0.61	2.191 ±0.018	0.456±0.04	996.03±5.11	2.8±0.02
CSR4	2.976±0.05	71.66±0.69	2.040 ±0.028	0.483±0.04	1068.04 ±8.38	2.8±0.05
CSR6	2.628±0.02	65.65±0.60	2.131 ± 0.026	0.461± 0.04	1005.88 ±6.91	2.8±0.05
CSR18	2.925±0.03	76.78±0.79	2.283 ±0.006	0.487±0.04	1157.04±6.21	2.6±0.03
CSR19	2.877±0.03	65.36±0.54	1.985 ±0.021	0.454±0.04	1001.28±9.62	2.5±0.05
NB7	1.826±0.02	72.78±0.69	1.588 ±0.003	0.294±0.03	749.72 ±5.16	2.1±0.04
NB18	2.196±0.04	73.45±1.13	1.593 ±0.004	0.286±0.03	728.80 ±10.56	2.3±0.04
CC1	1.936±0.02	64.44±0.51	1.677 ±0.008	0.298±0.03	661.87 ±6.56	2.1±0.04
CA2	1.867±0.01	65.61±0.59	1.625 ±0.005	0.285±0.03	681.43 ±5.11	2.3±0.03
PAM101	2.075±0.02	63.53±0.77	1.582 ±0.008	0.264±0.02	812.95 ±7.18	2.0±0.05
PAM111	2.088±0.02	71.09±0.94	1.596 ±0.013	0.354±0.03	889.33 ±10.51	1.8±0.02
P5	2.289±0.04	64.70±0.49	1.606 ±0.004	0.259±0.02	782.26±4.41	2.1±0.06
CSR2 x CSR4	2.872±0.03	70.20±0.76	1.886 ±0.003	0.379±0.03	953.85± 6.42	2.8±0.03
CSR4 x CSR2	2.846±0.01	72.64±0.82	1.892±0.004	0.427±0.04	979.93 ±4.59	2.8±0.05
SEd	0.0503	1.05	0.0178	0.0065	10.266	0.0563
CD value 0.05%)	0.0994	2.086	0.0352	0.0129	20.265	0.1122

\*Mean of ten replications ± SD

any other races. The hybrids CSR2 x CSR4 and CSR4 x CSR2 recorded 0.379 and 0.427 g respectively (Table 1 & 2), which was similar to the observations recorded by Basavaraja (2001).

**Table 3. Amylase activity in bivoltine and multi voltine silkworm races\***

Bivoltine Race	Amylase content (µg / 100µl/h )	Multivoltine Races	Amylase content (µg /100µl/h )
NB4D2	180.42 ± 1.21	Kolar Gold	1162.17 ± 14.82
SH6	131.53 ± 1.47	Kollegal Jawan	1325.05 ± 13.26
SF19	136.90 ± 1.45	C Nichi	1571.52 ± 10.59
CSR2	135.43 ±0.88	Saruput	1284.98 ± 8.7
CSR3	173.23 ± 1.23	P4D3	1442.38 ± 13.75
CSR4	174.33 ± 1.45	PA12	1200.90 ± 12.02
CSR6	148.63 ± 0.94	Tamil Nadu white	1064.85 ± 23.41
CSR18	123.23 ± 1.80	Hosa Mysore	1412.43 ± 11.41
CSR19	119.56 ± 1.18	Mysore Princess	1286.08 ± 21.54
NB7	122.13 ± 1.09	CB5	1648.85 ± 13.74
NB18	151.60 ± 0.50	Nistari	1722.54 ± 16.11
CC1	120.80 ± 1.02	Pure Mysore	1500.24 ± 6.66
CA2	141.20 ± 0.12		
PAM101	184.03 ± 0.88		
PAM111	137.63 ± 1.17		
P5	151.16 ±131		
CSR2 x CSR4	173.76 ± 1.49		
CSR4 x CSR2	174.15 ± 1.16		
SEd	1.657	SEd	20.6254
CD (0.05%)	3.362	CD (0.05%)	41.4723

\*Mean of ten replications ± SD

**Single filament length**

The single filament length in multivoltines ranged from 306.58 m (Pure Mysore) to 696.73 m (Kollegal Jawan). Among bivoltines, CSR races recorded significantly longer filament than other bivoltine races (Table 1 & 2). Similar results were observed by Ashwath *et al.* (2000) and Dandin *et al.* (2001), who recorded the single filament length of 1000 - 1400 m in CSR races.

**Denier**

Denier was less in multivoltines and higher in bivoltines. Fine denier was exhibited by 2.82 in Sarupat race, whereas Pure Mysore recorded 1.04 denier. Among bivoltines, CSR2 recorded the denier of 3.3 followed by CSR4 (2.9) and other CSR races CSR3, CSR6, CSR18 and CSR19 exhibited similar denier. The significantly lower denier was exhibited in SH6 (Table 1 & 2). The denier estimates were similar to that of Dandin *et al.* (2001) and Murugesh (2002).

**Digestive amylase activity**

The highest activity was recorded in Nistari followed by CA2, Pure Mysore, P4D3 and Hosa Mysore. Similar type of results was obtained by Chatterjee *et al.* (1992) where they analysed with haemolymph amylase. Higher digestive amylase in low yielding races and low digestive amylase in high yielding races exhibited in this study is well supported with findings from earlier studies

(Chatterjee *et al.*, 1992, Patnaik *et al.*, 1998). Kolar Gold recorded 1162.77g and Tamil Nadu white recorded 1054.85 g. Similarly, Patnaik *et al.* (1998) reported that digestive amylase converted about 280 g in 30 minutes in Mysore Princess race. The variation may be due to mulberry plant type, environment and rearing conditions. The results were similar in other bivoltine races like NB18, as reported by Patnaik *et al.* (1998). All the CSR races and hybrids except CSR2 (135.4 g) and CSR19 (119.56 g) recorded significantly equal activity. CSR4 (174.33 g), CSR3 (173.33 g), CSR2 x CSR4, (173.75 g) and CSR4 x CSR2 (174.15 g) were significantly

on par in amylase activity (Table 3). Similar results were obtained by Patnaik and Datta (1995).

#### **Correlation between amylase activity and biological and yield traits**

A correlation analysis between digestive amylase activity with larval weight, cocoon weight, pupal weight, shell weight, effective rate of rearing, single filament length and denier related to 30 silkworm races was taken up in this study. The positive significant correlation between digestive amylase activity and effective rate of rearing was recorded. Negative correlation between amylase activity and

**Table 4. Correlation of biological and yield related parameters with digestive amylase activity**

Parameter	Larval weight (g)	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Denier (D)	Hatching (%)	Single Filament Length (m)	Amylase activity ( $\mu\text{g}/100\mu\text{l/h}$ )	ERR (%)
Larval wt (g)	1.000								
Cocoon wt (g)	0.940**	1.000							
Pupal wt (g)	0.935**	0.994**	1.000						
Shell wt (g)	0.945**	0.977**	0.970**	1.000					
Denier	0.652**	0.605**	0.596**	0.626**	1.000				
Hatching (%)	0.178	0.126	0.131	0.128	0.159	1.000			
Single filament length (m)	0.923**	0.937**	0.929**	0.945**	0.566**	0.117	1.000		
Amylase Activity ( $\mu\text{g}/100\mu\text{l/h}$ )	-0.838**	-0.921**	-0.925**	-0.860**	-0.429**	-0.115	-0.850**	1.000	
ERR (%)	-0.664**	-0.745**	-0.752**	-0.665**	-0.388**	-0.059	-0.595**	0.812**	1.000

\*\* Significant at 1% level (2-tailed).

different biological and yield related traits were also recorded. For all the biological parameters, the negative correlation coefficients ranged from -0.838 to -0.925. The correlation between the denier and digestive amylase was also significant (Table 4). The correlation study made by Chatterjee *et al.* (1992) also revealed a significant positive correlation of digestive amylase activity with survival and significant negative correlation of digestive amylase activity with larval weight. Similarly, the results for other correlations obtained in the present experiment also went in accordance with Patnaik *et al.* (1998). The positive correlation between survival (ERR) and digestive amylase activity could be explained by the better digestion capacity in turn due to higher amylase activity. The traditional Indian races have 10-15 fold higher amylase activity than the high yielding bivoltine races. Similarly, 40 fold increase was reported earlier by Abraham *et al.* (1992). The negative correlation between yield related traits and digestive amylase activity gets support from the findings of Chatterjee *et al.* (1992) and Patnaik *et al.* (1998).

It can be concluded that the races with higher amylase activity possessed higher survival rate and lower economic parameters but reverse was evident in races with low amylase activity. Hence, crossing

both of them may result in higher silk productivity and survival rate.

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