



Quantitative Estimation of Amylase Activity in Selected Larval Mutants of Silkworm *Bombyx mori* L. Reared on Two Mulberry Varieties

M. Devamani and R.S. Umakanth*

Dept. of Studies in Sericulture Science, University of Mysore, Manasagangothri, Mysuru, Karnataka (570 006), India

Open Access

Corresponding Author

R.S. Umakanth

✉: umakanth@sericulture.uni-mysore.ac.in

Conflict of interests: The author has declared that no conflict of interest exists.

How to cite this article?

Devamani and Umakanth, 2023. Quantitative Estimation of Amylase Activity in Selected Larval Mutants of Silkworm *Bombyx mori* L. Reared on Two Mulberry Varieties. *Plant Health Archives* 1(2), 61-65. DOI: 10.54083/PHA/1.2.2023/61-65.

Copyright: © 2023 Devamani and Umakanth. This is an open access article that permits unrestricted use, distribution and reproduction in any medium after the author(s) and source are credited.

Abstract

Four larval mutants namely Knobbed, Zebra, pere and Ursa of the silkworm, *Bombyx mori* L. were selected for the present investigation. The digestive juice and haemolymph were collected from fifth instar larvae (day 1 to day 6) and the samples were subjected to spectrophotometric analysis for estimation of amylase activity. The recorded O.D. values (at 525 nm) were converted into maltose released ($\mu\text{moles ml}^{-1}$). The results of the present study clearly indicate that the amylase activity gets enhanced with the increase in number of feedings along with the advancement of age during 5th instar larval stage (day 1 to 6). Based on the results obtained, Zebra performed better followed by Knobbed than the other two larval mutants under study. The larvae fed with V_1 variety performed better than those batches fed with S_{36} variety of mulberry in both the samples. Of the two samples, amylase activity recorded higher in digestive juice than haemolymph in all the four larval mutants under study.

Keywords: Digestive juice, Haemolymph, Mulberry, Mutant silkworm, S_{36} , V_1

Introduction

The silkworm, *Bombyx mori* is a vital economic sericigenous insect that primarily consumes mulberry leaves and transforms leaf protein into silk protein. In silkworms, most of the traits responsible for silk yield are under the polygenic control (Lokesh *et al.*, 2012; Umakanth and Krishnamurthy, 2011). Enzymes play a vital role in the metabolism of dietary food in the body of an organism (Lokesh *et al.*, 2012), which is also true in the silkworm, *Bombyx mori* L. Studies on the genetic variability of amylase activity of Indian races and other exotic breeds of the silkworm, *B. mori* and its role on the expression of yield components (Chatterjee *et al.*, 1992; Thimmaraju, 2008) is an important field of study in biochemical genetics. Amylase is an enzyme that performs the function of catalysis by way of hydrolysis of starch into sugars and amylase is an important enzyme involved in the metabolism of carbohydrates in the silkworm (Patnaik and Datta, 1995). The amylase is utilized as a marker with the conventional phenotypic selection for improving the survival potential of productive breed for maximizing the yield. The

two forms of hydrolyses, alfa-amylase and alfa-glucosidase are key enzymes involved in starch breakdown and absorption respectively (Ashwath *et al.*, 2002; Ashwath *et al.*, 2009; Lokesh *et al.*, 2012). Current understanding suggests that inhibiting the enzymes responsible for carbohydrate digestion and absorption can have a substantial impact on the overall carbohydrate levels within the mid-gut and haemolymph (Ganie *et al.*, 2017). Studies were conducted to estimate amylase activity quantitatively in four larval mutants of the silkworm *Bombyx mori* L. reared using S_{36} and V_1 mulberry varieties.

Materials and Methods

Four mutant larvae viz., Ursa, Zebra, Knobbed and pere were reared using V_1 and S_{36} varieties of mulberry. The dfl's were procured from silkworm seed production centre (NSSO) Mysuru and were reared following the standard rearing procedure (Dandin and Giridhar, 2010) at Department of Studies in Sericulture Science, University of Mysore, Manasa Gangothri, Mysuru. Two popular mulberry varieties, viz., S_{36}

Article History

RECEIVED on 11th June 2023

RECEIVED in revised form 12th September 2023

ACCEPTED in final form 19th September 2023

and V_1 were fed to the silkworm larvae from first instar to fifth instar by dividing the larvae into two batches of three replicates each. The samples from the first day to sixth day of fifth instar larvae were collected into precooled eppendorf tubes (5 °C). The larvae were induced to vomit the digestive (gut) juice by brief exposure to chloroform vapours for collection of the sample and by cutting the fourth abdominal legs for the haemolymph collection. The collected samples were then centrifuged at 10,000 rpm for 10 minutes to remove undigested leaf particles in digestive juice; a pinch of thiourea was added to the sample tubes with haemolymph and was stored at -20 °C for further process in the experiment.

Estimation of Amylase Activity (Noelting and Bernfeld, 1948 Method)

2 ml of 0.2% starch and 20 microlitres of samples were added to each test tube and were incubated at 30 °C for 30 minutes on hot water bath. Then 2 ml of 3-5 DNS was added and boiled on water bath at 50-60 °C for 5 minutes and cooled immediately under running water, and the O.D. values of the end product was recorded by using spectrometer at 525 nm to calculate the amount of maltose released as $\mu\text{moles ml}^{-1}$ (Seetharamulu *et al.*, 2022). During the rearing data of selected economic traits namely hatching percentage, larval duration, cocoon weight, pupal weight, shell weight, shell ratio, filament length, filament weight, denier and renditta were collected to correlate the same with the amylase activity (Seetharamulu *et al.*, 2022).

Results and Discussion

Activity of Digestive Amylase

The activity of digestive amylase in the gut juice is presented graphically in figure 1 for all the four larval mutants selected for the present study. The activity of digestive amylase of Zebra & Knobbed mutants showed maximum amylase activity (148 and 142 μm) on fifth day of fifth instar fed with V_1 variety, followed by Ursa and pere (138 and 124 μm). On fourth day, Ursa and Zebra reported maximum amylase activity (138 and 134 μm). It is a well-known fact that, during the fifth instar of silkworm development, approximately 84% of the food is consumed dedicated to accumulating the necessary energy to support its metabolism during non-feeding stages (Ganie *et al.*, 2017). The obtained

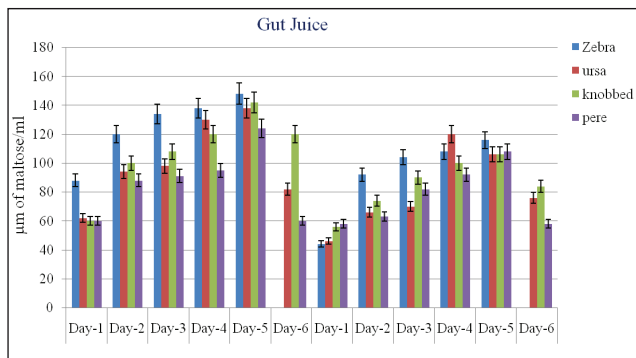


Figure 1: Graphical presentation of digestive amylase activity in larval mutants fed with V_1 and S_{36} variety

results are in agreement with the hypothesis that amylase enzyme contributes significantly in ingestion and digestion of mulberry leaf consumed (Farshid and Mahesha, 2012); hence, the activity of amylase will increase during the 5th instar larvae (Hirata and Yosuo, 1974).

The digestive amylase activity in the 5th instar larvae significantly varies among the larval mutants fed with V_1 and S_{36} mulberry varieties. There was a steep increase in the digestive amylase activity from third day onwards and maximum activity was observed on fifth day. The digestive amylase activity (fed with V_1 variety) was found maximum in Zebra (148 μm) and minimum in pere (124 μm) in fifth instar fifth day showed maximum activity. The digestive amylase activity (fed with S_{36}) was found maximum in Zebra (116 μm) and minimum (106 μm) in fifth instar larvae.

The purification of the digestive amylase was successfully executed in tasar silkworm (Nagaraju and Abraham, 1995). Positive correlation between yield and biochemical parameters was observed and significant negative correlations in some races (Chatterjee *et al.*, 1993) are seen based on the results obtained. Amylase activity has a direct relationship between the digestive juice and the quantitative traits correlated to understand its importance (Sharmila *et al.*, 2011).

Activity of Haemolymph Amylases

The activity of haemolymph amylase in the gut juice is presented graphically in figure 2 for all the four larval mutants selected for the present study. The amylase activity in the hemolymph of the silkworm mutants during fifth instar fluctuated according to their development (Ganie *et al.*, 2017; Sen *et al.*, 1991). The activity of hemolymph amylases fed with V_1 mulberry variety showed maximum in pere (94 μm) and minimum in Zebra (73 μm). The activity of hemolymph amylases fed with S_{36} variety showed maximum in pere (92 μm) and minimum in Zebra (58 μm). The general observation is that the amylase activity show increasing trend from third day to fifth day of fifth instar larvae and lesser compared to digestive amylase. The pere has recorded maximum activity in day 5 and 6 for both batches fed with V_1 and S_{36} variety, while Knobbed, Ursa recorded higher activity during day 2, 3, 4 and 5 but a gradual reduction was seen in day 6, of fifth instar. The haemolymph amylase activity

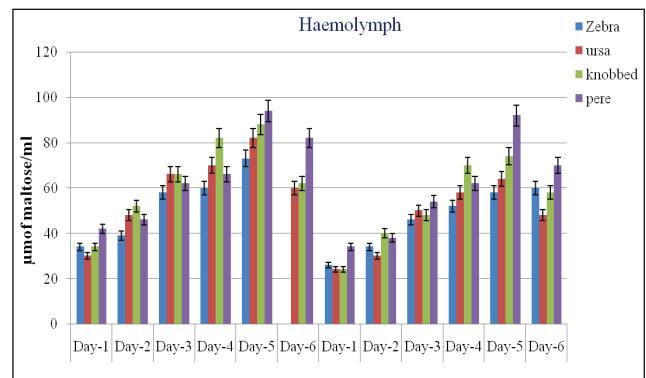


Figure 2: Graphical presentation of haemolymph amylase activity in larval mutants fed with V_1 and S_{36} variety

increases gradually from day 1 to day 5 followed by a slight decrease in day 6 similar to the digestive amylase. Among the two varieties fed to the larval mutants, the activity is higher in larvae fed with V₁ variety, than those fed with S₃₆ variety, similar reports were recorded by earlier workers (Tanaka and Kusano, 1980).

The larval mutants were selected for the present study because mutants serve as a good biological agent for various biochemical analyses and also to undertake fundamental studies of insect genetics. There are no efforts and reports where larval mutants of the silkworm *B. mori* were used for the biochemical studies, i.e., quantitative estimation of amylase activity, which has yielded interesting information, has been revealed based on the results of the present study. The amylase activity shows gradual increase with the advancement of age of the larvae during fifth instar from the day one to day five as it is directly involved in ingestion and digestion of mulberry leaf.

The amylase content is higher in the digestive juice than haemolymph among the two samples studied, and the present result is in agreement with the previous work undertaken and also reported (Umakanth and Devamani, 2016; Devamani and Umakanth, 2019). Amylase activity is reported to be high in the larvae fed with V₁ mulberry variety, which is highly recommended for feeding the silkworms to increase their silk productivity compared to that of S₃₆ variety, the same results were reported; hence it is popular among the farmers who practice sericulture. In addition, amylase activity is an important parameter

in the silkworm improvement programme following the contemporary breeding methods (Patnaik *et al.*, 1998).

Economic Traits

The mean values of the selected economic traits of four larval mutants of the silkworm, *B. mori* are presented in table 1. Among the four larval mutants selected for the study, Knobbed fed with S₃₆ showed higher cocoon, shell, pupal weight and filament length and lower values in Ursa fed with S₃₆. The pere larvae fed with V₁ variety leaf showed low cocoon, shell, pupal weight and filament length. In general, the larvae of Knobbed fed with S₃₆ showed higher values for economic traits than the larvae of other races fed with V₁. Profound biochemical changes occur in the insect body particularly by secretion of hormones and enzymes, which undergo variations during the growth and development. Many reports show that amylase activity gradually increases from day 1 to day 5 of the fifth instar larvae as the consumption of the leaf is around 80% with the advancement of the age of the larvae (Sowmya, 2012). It creates a suitable environment for absorption and its utilization of food consumed during growth (Seetharamulu *et al.*, 2022), leading to protein absorption by silkworm larvae gets accumulated in the silk glands. Chatterjee *et al.* (1992) has reported the importance of digestive amylase, which plays important role in the survival of the silkworm, by way of disease resistance. The activity of amylase will drop in the larvae as they mature and approach the spinning process with consumption and ingestion of the mulberry gets reduced gradually.

Table 1: Showing the mean values of various economic traits of four larval mutants of the mulberry silkworm, *Bombyx mori* L.

Larval mutants	Mulberry variety used	Hatching (%)	Larval duration (hr)	5 th instar single larval weight (g)	Single Cocoon weight (g)	Shell weight (g)	Pupal weight (g)	Shell ratio (%)	Average filament length (m)	Filament weight (g)	Denier (d)	Rend-itta (Kg)
pere	V ₁	88.49	600	4.10	2.10±0.23	0.610±0.01	1.51±0.08	21.71	999	0.772	4.08	7.99
	S ₃₆			4.38	2.14±0.30	0.609±0.05	1.54±0.05	22.23	1039	1.544	4.11	7.02
Knobbed	V ₁	94.39	600	4.89	2.62±0.11	0.753±0.05	1.50±0.03	23.08	1090	0.888	4.16	7.27
	S ₃₆			5.10	2.77±0.14	0.806±0.05	1.79±0.02	23.58	1099	1.150	4.20	7.19
Ursa	V ₁	91.57	600	4.21	2.32±0.15	0.554±0.04	1.81±0.01	21.88	1003	0.474	4.14	7.89
	S ₃₆			4.06	2.21±0.03	0.524±0.05	1.66±0.04	21.43	997	0.292	4.13	7.87
Zebra	V ₁	90.59	600	4.62	2.72±0.11	0.801±0.04	1.69±0.03	23.45	1042	1.460	4.21	7.96
	S ₃₆			4.14	2.64±0.31	0.784±0.03	1.54±0.02	22.97	1007	1.389	4.15	7.68

Conclusion

The amylase activity gradually increases with the advancement of age of the larvae during fifth instar from the day one to day five. The amylase content is higher in the digestive juice than that of haemolymph. The digestive amylase activity larvae fed with V₁ mulberry variety was found maximum in Zebra and minimum in pere and among the fifth instar, fifth day showed maximum amylase activity compared to those fed with S₃₆ mulberry variety. The activity of hemolymph amylases in the mutant larvae fed with V₁ variety, which recorded higher values in pere and lower in Zebra. In general, Knobbed larvae fed with S₃₆ have shown higher values for economic traits than those larvae fed with V₁ variety.

Acknowledgement

The first author would like to thank the Chairman, Department of Studies in Sericulture Science, University of Mysore, Manasa Gangothi, Mysuru for the facilities provided during the conduct of the study.

References

- Ashwath, S.K., Sreekumar, S., Toms, J.T., Dandin, S.B., Kamble, C.K., 2009. Identification of RAPD markers linked to digestive amylase genes using near isogenic lines of the silkworm, *Bombyx mori* L. *Journal of Insect Science* 10(1), 84. DOI: 10.1673/031.010.8401.
- Ashwath, S.K., Morrison, M.N., Datta, R.K., 2002. Evolution of bivoltine silkworm breeds by amylase isozyme selection and evaluation of hybrids. In: *Advances in Indian Sericultural Research (Pro. Nat. Conf. Strat. Seric. Res. Dev.; November 16-18, 2002)* (Eds.) Dandin, S.B. and Gupta, V.P. CSR&TI, Mysore. pp. 71-75.
- Chatterjee, S.N., Rao, C.G.P., Chatterjee, G.K., Ashwath, S.K., Patnaik, A.K., 1993. Correlation between yield and biochemical parameters in the mulberry silkworm, *Bombyx mori* L. *Theoretical and Applied Genetics* 87, 385-391. DOI: 10.1007/BF01184928.
- Chatterjee, S.N., Rao, C.G.P., Chatterjee, G.K., Ashwath, S.K., 1992. Genetic variability of amylase activity in the mulberry silkworm, *Bombyx mori* L. and its significance. *Sericologia* 32(4), 671-683.
- Dandin, S.B., Giridhar, K., 2010. *Handbook of Sericulture Technologies*. Central Silk Board, Bangalore. p. 420.
- Devamani, M., Umakanth, R.S., 2019. Quantitative and qualitative evaluation of effect of Dimethoate on amylase activity in silkworm *Bombyx mori* L. *Journal of Emerging Technologies and Innovative Research* 6(6), 96-107.
- Farshid, G.K., Mahesha, H.B., 2012. Analysis of correlation between haemolymph and mid-gut tissue amylase with commercial characters of silkworm *Bombyx mori* L. *Annals of Biological Research* 3(7), 3518-3532.
- Ganie, N.A., Kamili, A.S., Rashid, M., Khan, I.L., Dar, K.A., Zia-ul-Haque, S., 2017. Haemolymph amylase activity in some tropical and temperate breeds of mulberry silkworm, *Bombyx mori* L. *Journal of Experimental Zoology, India* 20(Part A), 227-232.
- Hirata, Yosuo, 1974. Relationship between the amylase activity of the larval digestive juice and several quantitative characters in a silkworm *Bombyx mori*. *The Journal of Sericultural Science of Japan* 43(5), 385-390. (in Japanese).
- Lokesh, G., Ananthanarayana, S.R., Murthy, V.N.Y., 2012. Changes in the activity of digestive enzymes in response to chemical mutagen diethyl sulfate in the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae). *Asian Journal of Applied Sciences* 5(6), 431-437. DOI: 10.3923/ajaps.2012.431.437.
- Nagaraju, J., Abraham, E.G., 1995. Purification and characterization of digestive amylase from the tasar silkworm, *Antheraea mylitta* (Lepidoptera: Saturniidae). *Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology* 110(1), 201-209. DOI: 10.1016/0305-0491(94)00121-A.
- Patnaik, A.K., Datta, R.K., 1995. Amylase - Its genetics and prospects as a marker in silkworm breeding. *Indian Journal of Sericulture* 34(2), 82-89.
- Patnaik, A.K., Ashwath, S.K., Datta, R.K., 1998. Relevance of biochemical parameters in the silkworm improvement programme. In: *Silkworm Breeding*. (Ed.) Reddy, G.S. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. pp. 253-270.
- Seetharamulu, J., Anitha, M., Jayashree, Alekhya, R., Madhavi, K., Raju, P.J., 2022. Studies on the amylase activity in the haemolymph of sex-limited silkworm *Bombyx mori* L. breeds for cocoon colour. *International Journal of Applied Research* 8(9), 17-21. DOI: 10.22271/allresearch.2022.v8.i9a.10111.
- Sen, R., Nagaraj, C.S., Suresh Kumar, N., Sengupta, K., 1991. Studies on the haemolymph amylase strain type in *Bombyx mori* L. frequency of amylase strain type in an experimental population of a few breeds of *Bombyx mori* L. *Indian Journal of Sericulture* 30(1), 38-41.
- Sharmila, K.K., Ashwath, S.K., Mahalingappa, K.C., Sudha, V.N., Thippeswamy, T., Nirmal Kumar, S., Qadri, S.M.H., 2011. Comparative analysis of amylase activity in the digestive juice of single hybrids evolved by amylase marker assisted selection. In: *Proceedings of National Conference on Sericulture Innovations - Before and Beyond*. January 28-29, 2011. Central Sericulture Research and Training Institute, Mysore. pp. 77-78.
- Sowmya, 2012. Studies on the quantitative estimation of amylase activity in selected bivoltine breeds/ hybrids of silkworm, *Bombyx mori* L. M.Sc. Dissertation, University of Mysore, Mysore. pp. 23-28.
- Umakanth, R.S., Krishnamurthy, N.B., 2011. Quantitative analysis of amylase profiles in two bivoltine breeds of the silkworm, *Bombyx mori* L. In: *Proceedings of National Conference on Sericulture Innovations - Before and Beyond*. January 28-29, 2011. Central Sericulture

- Research and Training Institute, Mysore. pp. 122-131.
- Umakanth, R.S., Devamani, M., 2016. Quantitative estimation of the amylase activity in larval mutants of the silkworm, *Bombyx mori*, L. In: *Recent Advances in Applied Biosciences*. (Eds.) Sobti, R.C., Jaiswal, K. and Mishra, S. Narendra Publishing House, New Delhi. pp. 71-79.
- Tanaka, Y., Kusano, T., 1980. The haemolymph amylase activity during development of the silkworm, *Bombyx mori* L. *The Journal of Sericultural Science of Japan* 49(2), 95-99. (in Japanese).
- Thimmaraju, K., 2008. Effect of fortification of mulberry leaves with poultry egg and botanicals on growth and development of silkworm, *Bombyx mori* L. M.Sc. Thesis, University of Agricultural Sciences, Bangalore.