STORAGE PROTEIN-2 AS A DEPENDABLE BIOCHEMICAL INDEX FOR SCREENING GERMPLASM STOCKS OF THE SILKWORM *BOMBYX MORI* (L.)

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Abstract

Storage protein (SP-2) variation was investigated among selected silkworm germplasm stocks representing two major potential sericulture areas of India. The expression levels of storage protein varied among them, as seen in Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), which correlated with their geographical origin. The storage protein variation is an inter origin variability and this differential expression of the protein is helpful to tag the robustness of the breed/race associated with parentage and their origin. Present study revealed that silkworm races/breeds viz., LMO, Kolar Gold and A4e possess higher protein content among the races studied. This may be correlated with their robustness reflecting higher survival rate in the varied environments prevailing in the tropical zone. Such identified races can be conserved as storage protein rich genetic stocks for their maximal genetic potentials and high-grade silk productivity.

Key Words: sericulture, parentage, protein expression, races, robustness, silk productivity

1. Introduction

The mulberry silkworm, *Bombyx mori*, is a major economic resource in many parts of India and has been raised for more than 5000 years in Asian countries. The silkworm has an open circulatory system containing haemolymph, which is the important depository of nutrition and energy [10]. The main haemolymph proteins, lipophorin (LP), storage protein (SP) and vitellogennin (Vg) are common in insects and have special functions for development, metamorphosis and reproduction [33]. Insect storage proteins are a class of proteins, which play an important role during the metamorphosis of insects [35, 39]. Storage proteins are the major reservoirs for amino acids that are utilized for cuticular proteins and tissue formation for adult development [19]. Lauffer [18] first reported the haemolymph proteins in the silkworm larvae, *B. mori*, as early as 1943. Storage proteins are known to occur in two forms in the silkworm, referred to as SP1 and SP2 [38]. SP1 storage protein is female specific protein and consists of a high proportion of methionine. SP2 is an arylphorin-type storage protein and is not female specific [4, 38, 22]. These storage proteins contribute to the growth of insects during their metamorphosis.

The storage proteins are mainly synthesized in large quantities by the fat body of actively feeding larvae and released into the haemolymph. At the conclusion of the feeding period, they are selectively taken up by the fat body cells and stored in the form of protein granules that are required for the development of the adult tissues [19, 29, 34]. SP1 is characterized by molecular weight of approximately 82 kDa and SP2 is characterized by molecular range of 72-76 kDa. SP1 consists of an exceptionally high content of methionine, while the amino acid composition of SP2 is analogous to the dipterous storage proteins, being rich in phenylalanine and tyrosine [38, 24, 23]. The mode of synthesis of storage protein in the larva varies in different insects. In *Manduca sexta* it was detected in the haemolymph of second instar larvae [23]. In silkworms, an enormous accumulation of the storage proteins occurs in the haemolymph at the final instar of the larvae [36]. The accumulated protein is estimated by an electrophoretic method, to detect the molecular weight and their staining intensities [5]. This procedure permits rapid and reliable identification of protein variations reflecting any genetic differences [32, 11].

Electrophoresis of proteins is a powerful tool for identification of genetic diversity and the Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is particularly considered a reliable technology, as the storage proteins are highly independent of environmental fluctuations. Various methods have been employed to estimate the amount of storage protein like ultra centrifugation data for

quantification of storage protein-1 [23], visual inspection of stained PAGE bands [14], densitometry of SDS-PAGE gels [31] and quantitative immunodiffusion to estimate the relative concentration of storage protein of *Hyalophora cecropia* and *Bombyx mori* [36, 38].

Estimation of storage proteins by SDS-PAGE gels is one of the easiest methods to quantify this protein [36, 38]. Earlier studies indicate a significant relation between storage proteins and metamorphic features viz., larval weight and pupation rate and also it has a special function on metamorphosis, which facilitates maximal growth after every moult in insects [19]. This specific functional utility of the storage protein as an index to measure the growth increment of different silkworm breeds and their better survivability as an indicator of silkworm breed robustness was reported by Somasundaram [33]

The present study focuses on the genetic variations in the expression level of storage proteins in various multivoltine silkworm breeds of Germplasm bank. Accumulation of storage protein in insects is greatly influenced by genetic factors [6]. The study highlights the storage protein identified in different silkworm races/breeds as a possible biochemical index to tag the silkworm traits, especially breed robustness, comprising of growth and survival factors. These two economic factors of *B. mori* are the important traits to select parental breed for their higher survival in any agro climatic zones.

2. Material and methods

Multivoltine silkworm stocks belonging to two different agro climatic regions of India viz., Karnataka and West Bengal were selected for the study. In Karnataka the climate is cold and warm whereas in West Bengal hot and humid conditions prevail. The multivoltine races selected for this study were reared in the Germplasm Centre at Hosur (latitude 12°45'N and longitude 77°5'E, altitude 942 M), Tamil Nadu India, following the standard rearing practices [16]. Ten races/breeds were selected for the study (Table 1). Ten larvae were randomly collected on the fourth day of fifth age and their haemolymph samples were drawn from the distal region of the prothoracic leg into prechilled eppendorf tubes containing phenylthiourea. The samples were centrifuged in RC5C Ultra centrifuge (Sorval Instruments) for five minutes at 12000 rpm for removing haemocytes and other tissue debris. 10 µl haemolymph from each multivoltine race was analyzed through spectrophotometer (Shimadzu, Japan) at 660 nm wave length. Total protein content was determined with folin reagent using Lowry method [21]. The quantity of protein was expressed as µg/ml.

Silkworm haemolymph was analysed by SDS-PAGE. The haemolymph extracts equivalent to 50 µg of protein were mixed with equal volume of Sodium Dodecyl Sulphate (SDS) sample buffer mixture (100% Glycerol, 10% SDS, 0.1% Bromophenol Blue, 1 ml of β-Mercapthoethanol and 2.5 ml of stacking buffer) and boiled for one minute. SDS-PAGE was performed using 6% stacking gel (Acrylamide, Bis acrylamide in the ratio of 30:0.8, Tris Buffer pH 6.8, 0.01% of Temed, 1.5% of Ammonium per sulphate and 0.05% of SDS) and 7.5% separating gel (Acrylamide, Bis acrylamide in the ratio of 30:0.8, Tris Buffer pH 8.8, 0.25% of Temed, 0.1% of SDS and 0.1% of Ammonium per sulphate). It was stained in Coomassive Brilliant Blue [17] (Coomassive Brilliant Blue 0.125%, Methanol 50% and Acetic acid 10%). The sample was run in tank buffer (0.025M Tris pH 8.3, 0.192 M Glycine, and 0.1% SDS) at 30V up to stacking and at 60V till the blue reached the bottom. The gel was stained with coomassive blue for 45 mins and washed with distilled water. Destained with 50% Methanol, 10% Acetic acid and 40 ml distilled water. The gel was stored in 7.5% Acetic acid. The Gel was observed under Gel Scanner (Syngene, USA). Storage protein band, SP-2 was identified by its position in the gel. Different density levels of the storage protein observed per unit area was interpolated against each accession. This data was subjected to 'Hierarchical Clustering' using Ward's Minimum Variance Clustering analysis from SPSS package, 11.5 version, which is a standard method to study relatedness and genetic diversity among breeds Clustering of the silkworm breeds thus obtained was analyzed.

3. Results

Among the ten multivoltine races studied, four races are from the state of Karnataka and six races from the state of West Bengal. The details of selected silkworm accessions, their parentage and origin are given in Table 1. The total protein content varied from 39 µg/ml to 17.0 µg/ml among the races studied. AP12 (BMI-0034) and CB5 (BMI 0021) showed the highest protein concentration and A4e (BMI-0032) showed the lowest protein concentration (Table-2).

The SDS-PAGE profile of these accessions showed remarkable variations in the staining intensity of SP2 proteins (Figure 1a & 1b), reflecting their origin and parentage. Total storage protein estimated among the ten races registered a highest staining intensity in LMO (BMI-0055) followed by Kolar Gold (BMI-0008).

Table 1. Details on Origin and Parentage of selected multivoltine races of *B. mori*

Sl. No.	Multivoltine accessions	Name of Breed	Parentage	Origin*	SP2 expression (Units/area)**
1	BMI-0008	Kolar Gold	(PM, NN6 (Hosho.Shungetsu)	KAR	1615030
2	BMI-0009	Kollegal Jawan	(PM, NN6 (Hosho.Shungetsu)	KAR	766623
3	BMI-0014	OS-616	Oval, S-15	WBL	749166
4	BMI-0016	G	N (X-ray), M2, KPG-B,C 1	WBL	741025
5	BMI-0021	CB5	N (M) M2, KB, N1 C110, C124, J124	WBL	1012183
6	BMI-0029	B	Nistari, KPG-B	WBL	723040
7	BMI-0032	A4e	(MYS1.BW) {M (N122.C110} (N124.C12	WBL	1600857
8	BMI-0034	AP12	A4e.PM	KAR	838625
9	BMI-0043	MW13	(PM.MP) NB7	KAR	599549
10	BMI-0055	LMO	Nistari.NistariM	WBL	2112616

Note: * Geographic area of the breed. KAR: Karnataka; WBL: West Bengal
**** Female specific protein (units/area)**

Table 2: Total Protein content in the haemolymph extracts of multivoltine races, *B. mori*

Sl. No	Multivoltine accession	Races/Breeds	Protein Conc. (µg/ml)
1	BMI-0008	Kolar Gold	35.2
2	BMI-0009	Kollegal Jawan	38.0
3	BMI-0014	OS-616	22.0
4	BMI-0016	G	34.0
5	BMI-0021	CB5	39.0
6	BMI-0029	B	36.9
7	BMI-0032	A4e	17.0
8	BMI-0034	AP12	39.0
9	BMI-0043	MW13	35.8
10	BMI-0055	LMO	26.7

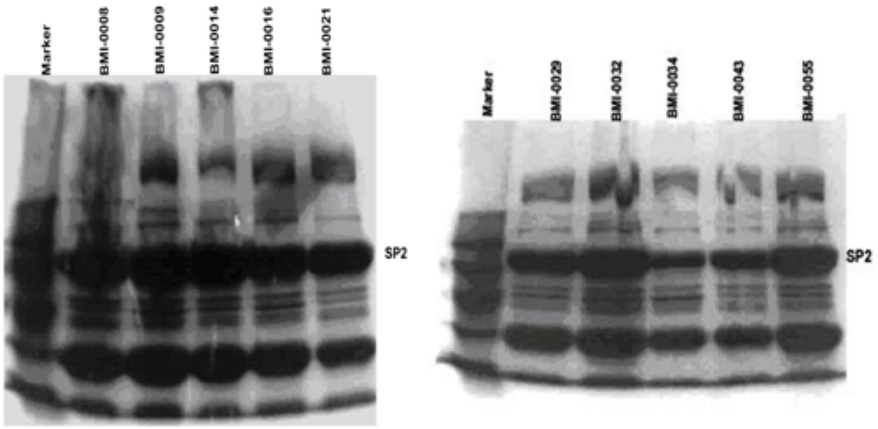

Figure 1: SDS-PAGE profile of SP2 in multivoltine accessions, *B. mori*

Lowest expression was found in MW13 (BMI-0043) followed by B (BMI-0029) (Table 3). The races were ranked 1 to 10, based on the differential expression of storage protein. The density of the protein expression was correlated with the pupation rate of the races. It was observed that the races with ranking 1-7, had pupation rate above 80% and the races that were ranked 8 to 10 had pupation rate below 80%.

A dendrogram generated by hierarchical clustering technique is shown in Figure 2. The resultant dendrogram showed four distinct cluster groups. First group comprises of three races viz., Kolar gold (BMI-0008), A4e (BMI-0032) and LMO (BMI-0055) (Table 4). These are possessing highest storage protein and further they are found in first cluster group. They share maximum percentage of geographical origin and parentage. This cluster group has its maximum origin from West Bengal and share parentage of Pure Mysore and Nistari, which are the hardy races of tropical climate. 75% similarity in sharing common origin has been found in this group. The second cluster comprises of two races viz., Kollegal Jawan (BMI-0009) and AP12 (BMI-0034) and the protein ranking are closely placed. Both of them share a common origin, Karnataka. Both are having Pure Mysore as one of the component in their parentage. Third cluster group comprises of two races viz., OS616 (BMI-0014) and CB5 (BMI-0021), which are having a common origin West Bengal. 80% similarity in sharing common origin has been found in this group. The fourth cluster group comprises of three races Viz., G (BMI-0016), MW13 (BMI-0043) and B (BMI-0029). Of these races two are from West Bengal and one from Karnataka, duly sharing 80% of common parentage (Table 4). Their SP2 ranking are very closely placed.

Table 3. Differential Expression of SP2, their ranking and pupation rate in the multivoltine races of *B. mori*

Multivoltine accessions	**Name of Breed**	**SP2 expression level (units/area)**	**Ranking of SP2**	**Pupation R (%)**
BMI-0008	Kolar Gold	1615030	2	80.24
BMI-0009	Kollegal Jawan	766623	6	87.28
BMI-0014	OS-616	749166	7	84.69
BMI-0016	G	741025	8	78.67
BMI-0021	CB5	1012183	4	80.81
BMI-0029	B	723040	9	71.20
BMI-0032	A4e	1600857	3	83.24
BMI-0034	AP12	838625	5	86.52
BMI-0043	MW13	599549	10	79.54
BMI-0055	LMO	2112616	1	84.36

Table 4: Grouping of *B. mori* races, based on Cluster analysis

Cluste	**Accessio**	**Races**	**Origin**	**Parentage**
I	BMI-000 BMI-003 BMI-005	Kolar Gold A4e LMO	KAR WBL WBL	(PM, NN6D)(Hosho.Shungetsu) (MYS1.BW){MYS (N122.C110} (N124.C12 Nistari.Nistari M
II	BMI-000 BMI-003	Kollegal jawan AP12	KAR KAR	(PM, NN6D)(Hosho.Shungetsu) A4e.PM
III	OS616 CB5	OS616 CB5	WBL WBL	Oval, S-15 N (M)M2,KB, N122, C110, C124, J124
IV	G MW13 B	G MW13 B	WBL KAR WBL	N (X-ray), M2, KPG-B, CB-1 (PM.MP) NB7 Nistari, KPG-B

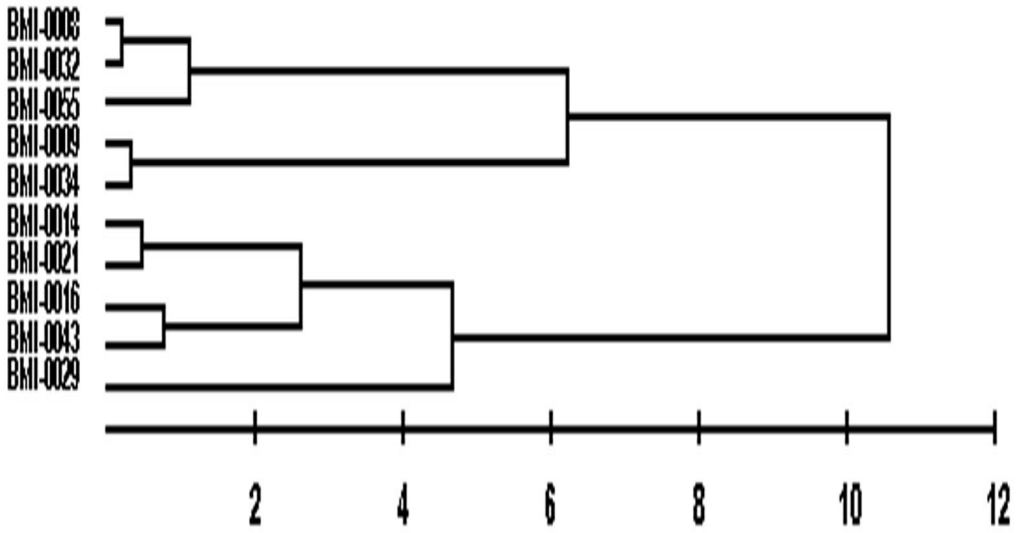

Figure 2: Cluster groups based on Wards Minimum Variance Clustering analysis

4. Discussion

Proteins have always been an interesting biochemical tool for insect biochemists because of their prominent role in development, morphogenesis and the intermediary metabolic pathways in insects. The proteins that are specifically synthesized in large amounts by fat body during last larval instar are designated as storage proteins [20]. Storage proteins are a major reservoir for growth and metamorphosis in insects in general and specifically in silkworms. The storage proteins are involved in moulting, metamorphosis, and cyclic reproduction. The transformation from larval caterpillar to non-feeding pupa and adult moth involves a complete remodeling and restructuring of the insect, and its organs [13, 9]. The maximum storage protein synthesis as measured from the staining intensity was observed in the haemolymph during larval life and subsequently declined during adult development has several postulates regarding their function. They act as a reservoir of amino acids to be utilized for the production of adult storage protein [26]. The storage proteins (SP1 and SP2) are synthesized abundantly in the peripheral fat body tissue and subsequently released into the haemolymph during the active feeding larval period of the silkworm. During the larval-pupal transformation, these proteins are sequestered by the perivisceral fat body and stored in crystalline form until they are utilized during pupal and adult development [8].

. Since proteins are the chief organic constituents of the cell, their macromolecules are concerned with the regulation of all the biochemical events in the organism [7]. Storage proteins are produced from the fat body during final instar of insect development [2]. The role of haemolymph and fat body in synthesis and storage of proteins in *B. mori* towards silk spinning is documented [37].

The total storage protein expression as seen from their staining intensity differed among the races studied. The races with similar geographical origin are placed in same group indicating the environmental impacts on the role of storage proteins. Higher pupation rate (>80%) was found in races exhibiting high staining intensity. This is in accordance with the observations on the changes in the concentration of primary metabolites especially total proteins corresponding to the changes noticed in the weights of larval body, silk gland, cocoon weight as well as the cocoon shell weight [30].

Densitometry scanning of bands relating to storage proteins exhibited wide variations in the staining intensity and this may be related to differential regulatory pattern of juvenile hormone (JH) in these accessions. Apart from this, the staining intensity of SP2 bands also differed much among origin and parentage. West Bengal origin and Karnataka origin accessions established a close relationship indicating close affinity in pattern of protein expression in spite of their different and varied climatic conditions.

In lepidopteron insects, synthesis of the storage protein is regulated by juvenile hormone suggesting that the regulation of storage protein synthesis by JH differs between races/breeds [3]. Exogenous juvenile hormone application in rice moth *Corcyra cephalonica* decreased the haemolymph storage protein levels suggesting that JH level alters the expression of storage protein [12]. Further, in insects, hormones control most of the developmental process directly or indirectly [1]. The observed variability in the synthesis of storage protein in the present study may be attributed to the difference in JH regulation in the silkworm breeds studied. This particular event of sequestration was characterized by a probable decline in the titer of Juvenile Hormone (JH) and an elevate titer of ecdysone [28]. These storage proteins are few in number and occur only in the larval stages where they accumulate in the haemolymph. Second, they are synthesized predominantly by the larval fat body and third, their concentration increases enormously in later larval instars especially in the last larval instar [19]. Synthesis of storage protein in the larval fat body is related with feeding activity [27] and the increase in amount of the storage proteins is evident during the mid feeding period of an instar. Further the quantity of storage protein does not increase during the larval moult or during starvation [25]. These observations suggest that the accumulation of storage protein is not only influenced by nutrional conditions but also by the ingrained genetic character of the races/breeds. The rate of development of an insect is dependent on the efficiency to feed, which is breed or race specific [6]. These observations suggest that storage protein accumulation in different races may be attributed to race specificity or genetic characteristics because of their different adaptability gained from their parental stocks.

For any directional breeding by breeders, to select the parents possessing high growth increment and higher survival rate, the storage protein index can be applied on a parental stock for choosing robust breed or race for commercial exploitation in silk industry.

Storage protein index, pupation rate index, were correlated and found that higher the SP2 storage protein higher the robustness as evidenced in the race LMO having pupation rate of above 80%. Hence storage protein can be used as a dependable Biochemical index to screen Germplasm stocks for robustness and for better utilization by breeders to develop a breed that can synthesize higher storage protein, for higher survival rate.

5. Conclusions

Storage proteins are found in the fat body at varied quantity specific to the requirement of each race adapted to different geographical regions. Such levels have been identified through SDS PAGE technique and scanning. Varied storage protein quantities observed in each race reflected on their genetic robustness, which is judged from certain quantitative traits like pupal growth. This study on storage protein measurement is a useful index to relate the robustness of race/breed for selecting genetically hardy parental stock for higher silk production. The study provides a clue to the role of storage protein in the geographical pattern of environmental impacts on metamorphosis and growth behavior. Further study in this line towards protein sequencing of this specific storage protein, which is underway, would amply indicate its intricate role on channelization of energy for growth variability and genetic diversity among germplasm stocks. Studies on storage proteins may be a dependable index for selecting the breeds for further multiplication of new breeds/lines in various silkworm breeds.

6. Acknowledgment

The authors acknowledge the services of Mr. S. Sekar, Assistant Director, Informatics Division, CSGRC, Hosur, India, for his assistance. Thanks are due to Dr. Srinivasababu, Silkworm Divisional Head, and Mr. Balachandran, Scientist C, CSGRC, Hosur, for providing the experimental material. Financial assistance from Central silk Board is gratefully acknowledged.

7. References

1. Calvez R: **Progress of developmental programme during the last instar of *Bombyx mori*; relationship with food intake, ecdysteroids and juvenile hormone.** *J Insect Physiol* 1981, 27: 233-239.
2. Chen PS: **Amino acid and protein metabolism.** *In: Comprehensive Insect Physiology, Biochemistry and Pharmacology.* 10. Eds. Kertkut G.A and Gilbert L I. 1985, pp.177-218. Pergamon Press, Oxford.
3. Chinzei Y, Azumi C, Miura K, Matsuoka H and Ando, K: **Changes in haemolymph proteins and**

their synthetic activities during development and engorgement and after juvenile hormone treatment in the kissing bug, *Rhodnius prolizus*. *J of Insect Physio* 1994, 40: 491-499.

4. Doira: **Developmental and sexual differences of blood proteins in the silkworm, *Bombyx mori*.** *Science Bulletein of the Faculty of Agriculture Kyushu University 1968*, 23: 205-214.
5. Hames BD and Rickwook D: **Gel electrophoresis of Proteins- A practical approach**, IRL press, Oxford 1984.
6. Hanumappa TV and Delvi MR: **Effect of temperature on food utilisation in different races of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)**. *Nutrional Ecology of Insects and Environment* 1989, pp.212-221.
7. Harper HA, Rodwell VM and Mayes PA: **Review of Physiological Chemistry**. Lange Medical Publication. Los Altos, California 1993, pp.31-182.
8. Haunerland NH and Shirk PD: **Regional and functional differentiation in the insect fat body.** *Annu Rev Entomol* 1995, 40:121-145.
9. Haunerland NH, Nair KK and Bowers WS: **Fat body heterogeneity during development of *Heliothis zea***. *Insect Biochem* 1990, 20: 829-837.
10. Hou Y, Zou Y, Wang F, Gong J, Zhong X., Xia Q and Zhao P: **Comparative analysis of proteome maps of silkworm hemolymph during different developmental stages**. *Proteome Science* 2010, 8: 45.
11. Hunter R and Market C: **Histochemical demonstration of enzymes separated by electrophoresis in starch gels**. *Science* 1957, 125:1294-1295.
12. Ismail SM and Dutta-Gupta S: **Juvenile hormone inhibits the synthesis of major larval haemolymph proteins in rice moth, *Corcyra cephalonica***. *Biochem Int* 1988, 17: 1093-1098.
13. Keeley LL: **Physiology and biochemistry of fat body. Structure of the fat body**. In: G.A. Kerkut and Gilbert (eds.), *Comprehensive Insect Physiology Biochemistry and Pharmacology*. Pergamon press 1985, London 9: 211-248.
14. Kinnear JF and Thomson JA. 1975. **Nature, origin and fate of major haemolymph proteins in *Calliphora***. *Insect Biochem* 5: 531-552.
15. Kramer SJ, Mundall EC and Law JH: **Purification and properties of manduca, an amino acid storage protein of the haemolymph of larval and pupal *Manduca sexta*.** *Insect Biochem*, 1980. 10: 279-288.
16. Krishnaswamy S: **New technology of silkworm rearing**, Bulletin No. 2, Central Sericulture and Training Institute: Mysore, India, 1978, pp. 1-23.
17. Laemmli UK: **Clevage of structural proteins during the assembly of the head of bacteriophage T4**. *Nature* (London) 1970, 227: 680-685.
18. Lauffer MA: **Ultra centrifugation studies on the blood of normal and jaundice diseased silkworm**. *Proc. Soc. Exp Bio Med* 1943, 52: 330-332.
19. Levenbook L: **Insect storage proteins**. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Kerkut, G.A and Gilbert, L.I (Eds). Pergamon Press, 1985, pp. 307-346.
20. Locke M and Collins JV: **The structure and formation of the protein granules in the fat body of an insect**. *J Cell Biol* 1965, 26: 857-885.
21. Lowry O, Rosebrough N, Faar A and Randall R: **Protein measurement with the Folin phenol reagent**. *J Biol Chem* 1951, 193: 265-275.
22. Mine E, Izumi S, Katsuki M and Tomino S: **Developmental and sex-dependent regulation of storage protein synthesis in the silkworm, *Bombyx mori***. *Dev Biol* 1983, 97: 329-337.
23. Munn EA and Greville GD: **The soluble proteins of developing *Calliphora erythrocephala* particularly caliphorin and similar protein in other insects**. *J Insect Physiol* 1969, 15: 1935-1950.
24. Munn EA, Feinstein A and Greville GD: **A major protein constituent of pupae of the blowfly *Calliphora erythrocephala* (Diptera).** *Biochem J* 1967, 102: 5-6
25. Nagata M and Kobayashi M: **Effects of nutrition on storage protein concentrations in the larval haemolymph of the silkworm, *Bombyx mori***. *J Seric Sci Jap* 1990, 59(6): 469-474.
26. Ogawa K and Tojo S: **Quantitative changes of storage proteins an vitellogene during the pupal-adult development in the silkworm, *Bombyx mori* (Lepidoptera L Bombicidae)**. *Appl Entomol Zool* 1981, 16: 288-296.
27. Riddiford L and Hice R: **Developmental profiles of the mRNAs for *Manduca arylphorin* and two other storage proteins during the final larval instar of *Manduca sexta***. *Insect Biochem* 1985, 15: 489-502.

28. Riddiford LM and Truman JW: **Biochemistry of insect hormones and insect growth regulators**. *Biochemistry of Insects* 1978, Ed. Rockstein, M, pp.308-357. Academic Press. New York.

29. Roberts DB and Brook HW: **The major serum proteins of Dipteran larvae**. *Experientia* 1981, 37: 103-110.

30. Sashindran Nair K, Sashindran Nair J and Vijayan VA: **Alteration in the primary metabolites in three different tissues of silkworm, *Bombyx mori* L. under the influence of a Juvenoid, R394**. *Caspian Journal of Environmental Sciences* 2007, 5: 27-33.

31. Sekaris CE and Scheller K: **Calliphorin, a major protein of the blowfly: Correlation between the amount of protein, its biosynthesis, and the titer of translatable calliphorin-mRNA during development.** *Dev Biol* 1977, 59: 12-23.

32. Smithies O: **Zone electrophoresis in starch gels: group variation in the serum proteins of normal individuals**. *Biochem J* 1955, 61: 629-641.

33. Somasundaram P, Krishnan M, Thangavelu K, Sinha RK, Ashok Kumar K and Kar PK: **Storage protein profiles in multivoltine silkworm breeds of *Bombyx mori* (L).** *Entomon* 2004, 29 (4): 323-329.

34. Telfer WH, Keim PS and Law JH: **Arylphorin, a new protein from *Hyalaphora cercropia*. Comparisions with calliphorin and manducin**. *Insect Biochem* 1983, 10: 289-303.

35. Thomson JA: **Major patterns of gene activity during development in holometabolous insects**. *Adv Insect Physiol* 1975, 11: 321-398

36. Tojo S, Betchaku T, Ziccardi VJ and Wyatt GR: **Fat body protein granules and storage proteins in the silkmoth, *Hyalophora cecropia*.** *J Cell Biol* 1978, 78: 823-838.

37. Tojo S, Kiguchi K and Kimura S: **Hormonal control of storage protein synthesis and uptake by the fat body in the silkworm *Bombyx mori***. *J Insect Physiol* 1981, 27 (7): 491-497.

38. Tojo S, Nagata M and Kobayashi M: **Storage proteins in the silkworm, *Bombyx mori***. *Insect Biochem* 1980, 10: 289-303.

39. Wyatt GR and Pan ML: **Insect plasma proteins**. *Annu Rev Biochem* 1978, 47: 779-817.