

Studies on the relationship between economic traits and Biomolecules of Mulberry Silkworm - A review

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Abstract

Mulberry silkworm *Bombyx mori* L. is playing an important role in the economic life of man since time immemorial. Though the conventional breeding techniques yielded several improved silkworm breeds with high productivity, have to overcome some limitations like genetic background, climatic influence, time duration *etc.* Recent advances in genetics and molecular biology *i.e.*, molecular markers have offered a number of alternative strategies to overcome the above limitations. Therefore, identification of suitable markers holds the key to successful implementation of marker assisted selection. Conventional library literature survey and online research databases with following key words *viz.*, correlation studies between (biomolecules) haemolymph, midgut, fat body, proteins, amylase, esterase, succinate dehydrogenase and (commercial characters) fecundity, hatching per cent age, weight of fifth instar larva, larval duration, cocoon weight, shell weight, shell ratio, filament length, denier and renditta were used. Review of literature showed that some authors have studied the correlation between selected characters of silkworm with selected biomolecules in selected silkworm breeds of both tropical and temperate regions. However, the clear evidence showing clear-cut correlation between biomolecules with commercial characters has not reported. This review identifies the workdone so far and reveals the gaps between the correlation studies. This reminds us for a technique/s for marker assisted selection of parents and progeny in silkworm breeding programmers for improved commercial characters. Further, it may be applicable in other crops in general.

Key words : Haemolymph, Midgut, Fat body, Proteins, Amylase, Esterase, Dehydrogenase.

Ever since its inception, more than four thousand years ago, Sericulture is playing an important role in the economic life of man²⁶. Due to their great economic value, more than 3000 genetically different silkworm (*Bombyx mori*) strains, are maintained in Europe and Asia³⁸, including India, the home land of all varieties of natural silk. To enhance the productivity and quality of silk fibers, many attempts are being made to improve the silkworm stocks through genetic manipulation. The findings of Chinese and Soviet breeders have revealed that the genetical and biometrical approaches to silkworm breeding will help in the evolution of suitable bivoltine breeds. In conventional breeding, the parental selection and performance prediction is on the basis of either their performance³⁷ or performance of the progeny^{2,8}. However, the traditional breeding approaches have to overcome some limitations like actual genetic basis of yield improvement, polygenic inheritance, environmental disturbances, interaction of genes including modifiers, occurrence of linkage drag and time duration. Recent advances in genetics and molecular biology have offered a number of alternative strategies to overcome the above limitations⁹. Silkworm breeders mainly concentrating on protein polymorphism in different races so as to provide insight into genetic variability between races to step up further hybridization programme to breed the best¹⁵. Thus, the study of polymorphic proteins of *Bombyx mori* is significantly important for selection and hybridization¹⁶. Recent advances in plant and animal breeding have highlighted the prospects of using linked molecular markers (isozyme/ DNA) for improvement of desirable traits. Therefore, identification of suitable markers

holds the key to successful implementation of marker assisted selection which is gaining ground fast in other fields of breeding⁶. A number of reports concerning the correlation aspects of silkworm *Bombyx mori* i.e., yield and biochemical parameters⁶; amylase and larval span, cocoon weight, shell weight, filament length, cocoon color, cocoon shape¹; Analysis of correlation between twelve selected commercial characters of silkworm *Bombyx mori*³²; Studies on the correlation between DNA, RNA and Proteins of *Bombyx mori*³³; Studies on the correlation between protein, amylase, succinate dehydrogenase, esterase and alkaline phosphatase of silkworm *Bombyx mori* L.³¹; Studies on correlation between haemolymph glucose level and commercial characters of *Bombyx mori* L.⁴³ have been published.

As the mulberry silkworm, *Bombyx mori* L. is the most important insect being used for commercial production of silk in sericulture industry; many attempts are being made to improve the silkworm stocks through genetic manipulation by conventional breeding techniques. However, conventional breeding programmes have their own limitations and marker assisted selection may exclude these limitation. With this background, various tissues were selected including haemolymph, midgut, fat body, digestive juice, whole animal tissues with important biomolecules like proteins, amylase, succinate dehydrogenase, esterase and alkaline phosphatase in all the above mentioned tissues were considered. The review of literature was carried out through conventional library reference work for previous work done before inception of e-literature facilities. For online searches Pub Med, Web of Science, Scopus, Science

Direct, Google Scholar, JSTOR *etc.*, are used. The key words used are the above mentioned biomolecules with selected commercial characters like fecundity, weight of fifth instar larva, larval duration, single cocoon weight, single shell weight, shell ratio, filament length, denier and renditta. After collection of all available literature, are analyzed for their applicability to consideration point of view and presented here to identify the workdone so far as well as gaps to fulfill the same.

The various aspects of protein metabolism including quantitative changes and metabolic activity of specific enzymes have attracted the interest of many insect biochemists. The available results from these biochemical studies indicated that the protein metabolism is of considerable importance in characterizing different stages of insect development⁷. Although our knowledge of insect haemolymph proteins has greatly advanced during the last decade, still only the origin and function of few major proteins are known⁴⁰. Obviously, the intensification of the protein metabolism is of paramount importance for the fifth instar larvae. However, the correlation studies between silkworm proteins and economic traits are scarce. Farshid and Mahesha¹³, studied correlation between haemolymph and midgut tissue proteins with commercial characters *viz.*, fecundity, larval weight, larval duration, cocoon weight, shell weight, shell ratio, filament length, denier and renditta in six silkworm breeds. The results clearly showed that the haemolymph protein has positive correlation with selected commercial characters except larval duration and renditta. Also, the midgut protein indicated positive correlation except fecundity, larval weight, shell ratio and renditta.

Mahesha and Farshid²⁹ reported that the fat body proteins showed moderately positive correlation with larval duration, cocoon weight, shell weight, shell percentage, filament length and denier. Though the above reports indicated the positive correlations between biomolecules and commercial characters, the number of breeds considered appears to be less in number. So, a clear-cut supportive literature about the relationship between biomolecules and commercial characters is obscure.

The α -amylases (α -1, 4-glucan-4-glucanohydrolases; EC 3.2.1.1) are the hydrolytic enzymes and are one of the key enzymes involved in digestion and carbohydrate metabolism in insects. Of the various enzymes analyzed, amylase is the most well worked out because of its association with economic characters of silkworm *Bombyx mori*³⁰. Hirata and Yosuo²³ found that the silkworm strains having more amylase activity, showed better cocoon weight, shell weight, shell percentage and cocoon productivity. Gamo¹⁷ reported that the *B. mori* strains with high amylase activity showed higher growth, economic traits, and survival than the low activity strains. Digestive amylase has been identified as a useful marker for breeding in the silkworm *Bombyx mori* L., due to its wide genetic divergence and its role in better digestibility and robustness³. Studies on relationship between the amylase activity and quantitative characters in *Bombyx mori* revealed that silkworm strains which have more amylase activity showed better cocoon weight, shell weight and shell percentage, and that the rate of synthesis of amylase is dependent upon rate of feeding. In silkworm, *Bombyx mori* and many other insect species successful adaptation depends on the level of digestive

amylases³⁰. Also, high basic condition in alimentary tract of caterpillars causes the enzyme very specific and favorable in case of biochemistry of digestion in the silkworm, *Bombyx mori*. The activity of *Bombyx mori* α -amylase was studied in both polyvoltine and bivoltine races. The polyvoltine races have adapted to tropical climate zones and exhibit high survival rate and short rearing time, although their silk fibers are short (500-700 meters) and of poor quality. Conversely, the bivoltine races have adapted to temperate zones and produce longer (1200-1500 meters) and higher quality silk fibers. However, the bivoltine breeds are weak and susceptible to diseases when reared in tropical climate. Interestingly, the activity of α -amylase in the digestive fluid of the polyvoltine races was higher than that of the bivoltine races, although the activity in their haemolymph did not differ. It was suggested that the increased enzyme activity of the polyvoltine races might be an adaptation to survive better in the tropical conditions¹. Moreover, the activity of the α -amylase in digestive fluid was higher than that in the haemolymph¹. Hirata and Yosuo²⁰, found that the silk worm strains which have more digestive amylase activity had better cocoon weight, shell weight and shell percentage. Chatterjee *et al.*,⁶ also, reported the importance of digestive amylase activity for the survival of the silkworm. Further, correlation studies between four yield attributes and six biochemical parameters among 54 *B. mori* strains has indicated close association of digestive amylase activity with survival, which was not affected by other enzymes Chatterjee *et al.*,⁶. These studies had clearly shown the prospects of using digestive amylase as a marker in *B. mori* breeding due to its wide genetic diversity,

its role in better digestibility, and higher survival⁹. Farshid and Mahesha¹⁴, studied correlation between haemolymph and midgut tissue amylase with commercial characters *viz.*, fecundity, larval weight, larval duration, cocoon weight, shell weight, shell ratio, filament length, denier and renditta in six silkworm breeds. The results clearly showed that the haemolymph amylase has highly positive correlation with selected commercial characters except larval duration. In contrast, the midgut amylase indicated highly positive correlation with larval duration only. Mahesha and Farshid²⁹, fat body amylase activity levels showed very low level of positive correlation filament length only selected silkworm varieties.

The succinate dehydrogenases (succinate: acceptor oxidoreductase; EC 1.3.99.1) is an enzyme complex, bound to the inner mitochondrial membrane of mammalian mitochondria, insects and many bacterial cells. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain and the activity levels may be correlated with the level of oxidation in a particular tissue. There is a considerable knowledge about the composition, enzymology and membrane binding of the enzyme and relatively new discoveries about its genetics and biosynthesis^{4,5,28}. In silkworm *Bombyx mori* most of the studies on the succinate dehydrogenase are limited and correlation studies are scarce. Recently, Ghasemi and Mahesha¹⁸ reported the correlation between Succinate dehydrogenase of haemolymph, midgut and fat body tissues with commercial characters *viz.*, fecundity, larval weight, larval duration, cocoon weight, shell weight, shell ratio, filament length, denier and renditta in six silkworm breeds. The results of

statistical analysis clearly showed that the haemolymph and midgut SDH activity level has positive correlation with selected commercial characters except renditta. Mahesha and Farshid²⁹, the SDH activity levels showed moderately positive correlation with renditta only.

Esterase (Acetylerases; EC 3.1.1.6) are a group of enzymes which catalyze the hydrolysis of various types of acetyl esters. Among the various isozymes, esterases have been studied extensively since they are the group of enzymes involved in metabolic and defense functions and are found in both soluble and membrane bound forms. The establishment of suitable biochemical markers for analyzing the degree of genetic heterogeneity may be used for a preliminary evaluation. Also they can be used for selection of the different breeds when making optimal variants for cross-breeding with reference to improving the most important economic signs and increasing the effectiveness of heterozygous selection⁴². When studying different breeds raised mainly in Japan, China, Korea, India and the former Soviet Union, in the group of nonspecific esterases from different tissues, a genetically determined polymorphism has been ascertained^{11,21,25}. The breeds of mulberry silkworms and their hybrids raised in Bulgaria have not been studied for analyzing the nonspecific gut esterases and finding out an eventual polymorphism⁴². Gillespie and Kojima²⁰ reported a relationship between level of polymorphism and metabolic enzymes such as esterase, in which are not involved in glycolysis and the citric acid cycle, than other enzymes involved in energy metabolism. The level of insect esterase highly variable

depending on the life stage, sex, tissue, hormones, strain, food, environmental conditions and numerous other factors¹⁰. Hendry *et al.*,²² stated that the role of esterase in the hindgut tissue of *Schistocerca gregaria* (Forsk.) is unclear, but since the hindgut tissue plays a role in excretion; it is conceivable that the carboxyl esterase is involved in the hydrolysis of metabolically inert and undesirable esters. The elevation of the esterase activity in the hindgut tissue might be also attributed to the presence of bacteria in the gut locust. Nevertheless, midgut esterases play a role in mosquito *Culex tarsalis* resistance to insecticide⁴⁵ and to allelochemicals²⁷. On the other hand, in the study on *Schistocerca gregaria* (Forsk.), the occurrence of both carboxylesterases and phosphotriesterases in the ovary tissue of 13-day-old and the presence of carboxylesterases in 2-day-old females might explain the correlation between ovarian maturation and occurrence of esterases as mentioned by Krishnamurthy and Umakanth²⁵ and Shaurub *et al.*,⁴¹. Staykova *et al.*,⁴² ascertained that the gut spectrum in silkworm comprises the greatest number of fractions, thus confirming the data of other authors^{12,36}. Ghasemi and Mahesha¹⁹ reported that the haemolymph esterase activity levels exhibited positive correlation with denier, filament length, larval duration, shell ratio, cocoon weight and shell weight. The midgut esterase activity levels showed positive correlation with denier, fecundity, filament length, larval weight, shell ratio, cocoon weight and shell weight. Also, the activity levels of fat body esterase indicated positive correlation with denier, fecundity, filament length, larval weight, shell ratio, cocoon weight and shell weight only. Also, the activity levels of fat body esterase indicated

positive correlation with denier, fecundity, filament length, larval weight, shell ratio, cocoon weight and shell weight only.

Alkaline phosphatases (Phosphoric monoester hydrolase; 3.1.3.1) are metalloenzymes, nonspecific, phosphomonoesterases³⁹, which exists in various organisms, involving as mediators in the energy transfer wherever ADP and ATP are involved in metabolic pathways. The activity level during various stages of ontogeny reflects the generation and utilization of energy. In insects, alkaline phosphatases are involved in several biological processes and responds to stress, pathogenesis or infection⁴⁴. The enzyme is located in the midgut, Malpighian tube, muscles, nerve fibers, and silk glands of the silkworm²⁴. The activity of the enzyme is related to the physiological condition of silkworms and reflects the digestion, absorption, and positive transport of nutrients in the midgut³⁴. Farshid and Mahesha³⁵ showed that the results of regression analysis clearly showed that midgut alkaline phosphatase has positive correlation with fecundity, larval duration and renditta only. The activity levels of fat body alkaline phosphatase showed positive correlation with fecundity, larval weight and renditta only.

Overview :

Several authors from both tropical and temperate regions have published some research work on correlation studies in silkworm. Their reports have clearly indicated the positive correlation between selected biomolecules and commercial characters. However, few limitation observed in these articles are number of silkworm breeds used

in their research work are less; the silkworms belongs to either tropical or temperature climatic conditions; the silkworm belongs to different voltine groups exhibited contrast results leading to confusions *i.e.*, bivoltines of temperate climate showed positive correlation with digestive amylase with cocoon characters. However, multivoltine groups of tropical climate did not exhibited positive correlation and positive correlation with resistance. Hence, these gaps may be considered.

Though several correlation studies between various biomolecules and commercial characters are reported from the silkworm *Bombyx mori* L. of both tropical and temperate breeds, no report showed clear – cut evidence to identify the biochemical and/or molecular markers. This might be due to variation in climatic conditions under tropical and temperature conditions, in addition to genetic makeup of the breeds selected. So this article tries to identify the gaps and indicates the necessity of suitable biomolecules for marker assisted selection. Hence, identification of biochemical markers for selection of parents as well as progeny during silkworm breeding programme under tropical climatic conditions is the need of sericulture field.

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