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CORRELATION BETWEEN BIOMOLECULES AND COMMERCIAL CHARACTERS OF SILKWORM *BOMBYX MORI* L. - 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, have to overcome some limitations. Recent advances in genetics and molecular biology have offered a number of alternative strategies to overcome the above limitations. Recent advances in plant and animal breeding have highlighted the prospects of using linked molecular markers for improvement of desirable traits. Therefore, identification of suitable markers, holds the key to successful implementation of marker assisted selection.

Keywords: Haemolymph, Midgut, Fatbody, Proteins, Amylase, Esterase

INRODUCTION

Ever since its inception, more than four thousand years ago, Sericulture is playing an important role in the economic life of man **[1]**. Due to their great economic value, more than 3000 genetically different silkworm (*Bombyx mori*) strains, are maintained in Europe and Asia **[2]**, 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. During 1980s, in addition to the application of quantitative genetics, several biotechnological and molecular approaches through quantitative trait locus are utilized for the success of the animal breeding programme initiated by the breeders. In conventional breeding, the parental selection and performance prediction is on the basis of either their performance **[3]** or performance of the progeny **[4, 5]**. 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 limitations **[6]**. 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 best **[7]**. Talebi *et al.*, **[8]** reported that the protein polymorphism gives a clue on the heterosis expression for selected traits and can be used as an index in silkworm breeding. Thus, the study of polymorphic proteins of *Bombyx mori* is significantly important for selection and hybridization **[9]**. 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 breeding **[6]**. A number of reports concerning the correlation aspects of silkworm *Bombyx mori* (esterase and cocoon shape **[10]**; esterase and ontogeny **[11]**; yield and biochemical parameters **[12]**; genetic variability for egg characters **[13]**; pupal size to fecundity and silk yield **[14]**; larval silk gland and shell weight **[15]**; amylase and larval span, single cocoon weight, single shell weight, filament length, cocoon color, cocoon shape **[16]** have been published.

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 development **[17]**. The proteins perform multiple functions in insects **[18]**. Krishnaswami *et al*., **[19]** observed that the increase in the protein concentration in the silkworm body after the fourth moult is due to regular feeding and substantial increase in

the body weight by the time the larva attains spinning stage. It is also reported that, the concentration of protein in silkworms increases progressively during larval development and reaches maximum in the late fifth instar larvae. Haemolymph proteins play an important role in insects for transport functions, as well as for their enzyme action. The synthesis and utilization of haemolymph proteins are controlled by genetic and hormonal factors **[20]**. 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 known **[21]**. The haemolymph proteins are implicated in ecdysis, growth of reproductive organs and salivary glands, formation of haemocytes and chitin **[18, 22]**. Obviously, the intensification of the protein metabolism is of paramount importance for the fifth instar larvae. Farshid and Mahesha **[23]**, studied correlation between haemolymph and midget 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 Farshid **[24]**, reported that the fat body proteins showed moderatively positive correlation with larval duration, cocoon weight, shell weight, shell percentage, filament length and denier.

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 **[25, 26]**. Of the various enzymes analyzed, amylase is the most well worked out because of its association with economic characters of silkworm *Bombyx mori* **[27]**. Hirata and Yosuo **[28]** found that the silkworm strains having more amylase activity, showed better cocoon weight, shell weight, shell percentage and cocoon productivity. Gamo **[29]** 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 robustness **[30]**. 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 amylases **[28]**. Bandani *et al*., **[31]**, stated amylases are one of the most important enzymes in digestive biochemistry of Lepidoptera because this order includes destructive herbivores. 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*. In the silkworm, *Bombyx mori* L., there are two sources of α- amylases, the digestive fluid and the hemolymph **[32]**. However, the role of haemolymph amylase is not yet known **[33]**. According to Wyatt **[34]**, it may participate in the degradation of glycogen in haemolymph. Nielsen *et al*., **[35]** stated that of the starch-hydrolysing enzymes, the α-amylases are of special importance as they are responsible for the solubilisation of starch. The activity of *B. mori* α-amylase (*Bm*AMY) 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 races are weak and susceptible to diseases when reared in tropical zones **[36]**. 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 hemolymph 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 conditions **[16]**. Moreover, the activity of the α-amylase in digestive fluid was higher than that in the hemolymph **[16, 37]**. Hirata and Yosuo **[28]** found that the silk worm strains which have more digestive amylase activity had better cocoon weight, shell weight and shell percentage. Chatterjee *et al*., **[12]** 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 **[12]**. 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 its higher survival **[6]**. Farshid and Mahesha **[38]**, studied correlation between haemolymph and midget 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 Farshid **[24]**, 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 acivity 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 **[39, 40]**, and relatively new discoveries about its genetics and biosynthesis **[41-43]**. In silkworm *Bombyx mori* most of the studies on the succinate dehydrogenase are limited to enzyme activity levels during cytoplasmic polyhedrosis **[44]**; in F1 progeny raised from ethyl methanesulfonate treated larvae **[27]**; when silkworms exposed to organophosphorous insecticides **[45]** and during uzi infestation **[46]**. Recently, Ghasemi Kasmaei and Mahesha **[47]** 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 Farshid **[24]**, the the SDH activity levels showed moderately positive correlation with rendita only.

Esterase (Acetylesterase; 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. Esterase isozyme exhibited higher-level of polymorphism in vertebrates and invertebrates **[48, 49]**. 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 selection **[50]**. With this regard different polymorphous enzyme groups have been studied **[51-55]**. 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 **[56-58]**. 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 polymorphism **[50]**. Gillespie and Kojima **[59]** 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. High level of allozyme diversity for esterases may also be related to greater variability of substrates and many of which are of external origin **[60]**. The level of insect esterase highly variable depending on the life stage, sex, tissue, hormones, strain, food, environmental conditions and numerous other factors **[61]**. Hendry *et al*., **[62]** stated that the role of esterase in the hindgut tissue of *Schistocerca gregaria* (Forskal) is unclear, but since the hindgut tissue plays a role in excretion; it is conceivable that the carboxylesterase 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 insecticide **[63]** and to allelochemicals **[64]**. On the other hand, in the study on *Schistocerca gregaria* (Forskal), 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 Umakanth **[58]** and Shaurub *et al*., **[65]**. Staykova *et al*., **[50]** ascertained that the gut spectrum in silkworm comprises the greatest number of fractions, thus confirming the data of other authors **[66, 67]**. Farshid and Mahesh **[68]**, 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, nonspecifc, phosphomonoesterases **[69]** which exists in various organisms from bacteria to mammals **[70-72]**, 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. The alkaline phosphatase has been characterized in holometabolous insects by many workers, including Denuce **[73]**, Rockstein **[74]**, Barker and Alexander **[75]**, Ashrafi **[76]**, Hodgson **[77]**, Sridhara and Bhat **[78]**, Lambremont and Schrader **[79]**, and Raychaudhri and Butz **[80]**. In insects, alkaline phosphatases are involved in several biological processes and responds to stress, pathogenesis or infection **[81]**. The enzyme is located in the midgut, Malpighian tube, muscles, nerve fibers, and silk glands of the silkworm **[82]**. Hegde and Krishnamurthy **[83]** and Raje Urs **[10]** analyzed phosphatases in some silkworm races. Umakanth **[11]** studied stage-specific, sex-specific, pupal- specific and moth- specific bands of phasphatases. Several factors affect the activity of ALKP in the midgut of silkworm, such as viral and bacterial infection and administered chemicals **[84]**. 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 midgut **[85, 86]**. Farshid and Mahsha **[87]**, 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.

The analysis of enzymes like amylase, succinate dehydrogenase **[27, 38, 47, 88]**, esterase, alkaline phosphatase **[68, 87, 89]** and alkaline protease **[90]** may help in silkworm breeding programme for cocoon characters and disease resistance. Hence, biochemical markers either qualitative or quantitative will help in the selection parents as well as progeny.

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