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Expression Profiling of Fibroin Gene in Various Strains of Bivoltine Silkworm, *Bombyx mori* L

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Abstract
Silk production in the mulberry silkworm, *Bombyx mori* L., is primarily governed by the synthesis and expression of fibroin genes in the posterior silk gland. Fibroin, the major structural protein of silk, consists of heavy chain (Fib-H), light chain (Fib-L), and P25 proteins, whose coordinated expression determines silk quality and productivity. The present review summarizes the current knowledge on fibroin gene structure, regulation, and expression profiling in silkworms, with particular emphasis on bivoltine strains of *B. mori*. Studies conducted using molecular, biochemical, transcriptomic, and proteomic approaches have demonstrated that fibroin gene expression is tightly regulated in a spatially and temporally specific manner, particularly during the fifth larval instar. Several investigations have revealed significant variation in fibroin gene expression among silkworm strains, which contributes to differences in cocoon traits, silk yield, and fiber quality. Advances in gene expression analysis, including quantitative PCR, RNA sequencing, and proteomic profiling, have provided valuable insights into the molecular mechanisms underlying silk protein biosynthesis. Understanding the expression dynamics of fibroin genes can facilitate the identification of superior silkworm genotypes with enhanced silk-producing potential and support the development of improved breeding strategies. The information compiled in this review highlights the importance of fibroin gene expression studies for genetic improvement, conservation of germplasm resources, and enhancement of silk productivity in bivoltine silkworms.

Keywords: *Bombyx mori*, fibroin gene, gene expression, posterior silk gland, bivoltine silkworm, silk protein

Introduction
The domesticated silkworm is not only an economically important insect for the silk production but has also been identified as the best model organism for lepidopteran biochemical, molecular genetics and genomic studies (Nagaraju and Goldsmith 2002) [21] with its known developmental and physiological characters. Additionally, *B. mori* has been used as an important bioreactor for the production of recombinant proteins (Tomita *et al*., 2003) [30]. The economic and scientific significance of the silkworm has made it the subject of intensive genetic studies since 20th century. Silkworm is a very good choice amongst laboratory species for research programmes. For domestication of *B. mori*, thousands of strains have been developed and maintained (Goldsmith *et al*., 2005) [7]. More than 400 mutations have been recorded in the silkworm gene bank and genetics and important molecular biology aspects have been studied extensively (Nagaraju, 2000) [20].
Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety or population of a species. The genetic diversity of *B. mori* is derived from hybridization of different geographical origins, mainly the Japanese, Chinese, European, and Indian strains, which have distinct traits. Silkworm breeds vary in their characteristic features and there exists a great deal of variability among various silkworm breeds. Therefore, it is imperative to have knowledge on the genetic make-up of silkworm breeds as it is a pre-requisite for choosing parents for hybrids which in turn results in the elevation of quantitative and qualitative aspects of cocoon production.
The process of silk synthesis and secretion has been studied extensively in *B. mori*. Silk from *B. mori* consists of two components, sericin and fibroin. Silk gland is the most important sub

organ responsible for the synthesis of silk core fibroin proteins in silkworm. Sericin makes up the protein coating of the silk filament and is secreted into the lumen from cells of the middle division of the silk gland. At least three silk genes are specifically expressed in the posterior, five other genes in middle, silk gland. Fibroin, the main part of the silk, is produced in the posterior region of the silk gland and contains three components: Fibroin-H chain, Fibroin-L chain, and Fibro hexamarine/ P25 (Tanaka and Mizuno; 2001) [29]. These three proteins form a hexameric complex with a ratio of 6:6:1 of Fib-H/Fib-L/p25 in the silk assemblage. Recently, increasing interest towards gene expression analysis has been towards to the structure of silk fibroin in connection with the biosynthesis of silk protein. Gene expression is the process by which information from a gene is used in the synthesis of functional gene product (Crick, 1958) [4]. These products are often proteins, but in non-protein coding genes such as transfer RNA (snRNA) genes, the product is a functional RNA. Gene expression process may be modulated/regulated, including the transcription splicing, translation, and post translational modification of protein. Regulation of gene expression gives control over the timing, location and an amount of given gene product (protein or ncRNA) present in cell and can have profound effect on the cellular structure and function. The products of genes active in posterior silk gland (PSG) include fibroin and p25 protein. PSG genes as well as the ser-1 gene differing in structure, exhibits a striking degree of homology of their 5′flanking sequences. This suggests a common regulatory mechanism. The expression of silk protein gene is probably controlled by tissue specific and general transcriptional factors. Hormones seem to participate in the regulation of expression of silk protein genes.

Functional genomics research has revealed information about the intricate mechanism regulating gene expression with both applied and theoretical significance. In this context, the present investigation was proposed with the aim to ascertain genetic diversity at molecular level among different bivoltine silkworm *Bombyx mori* L. strains based on their fibroin gene expression which is expected to identify silkworm strains with higher fibroin synthesizing potential and can be used in future breeding programmes to develop silkworm breeds/Hybrids for better cocoon and silk productivity.

Although considerable progress has been made in understanding the molecular structure, regulation, and biosynthesis of fibroin proteins in *Bombyx mori*, information regarding the differential expression of fibroin genes among bivoltine silkworm strains remains limited. Most studies have focused on developmental regulation and characterization of fibroin genes, whereas comparative expression analyses across commercially important bivoltine genotypes have received relatively less attention. Furthermore, the association between fibroin gene expression and economically important traits such as cocoon yield, shell weight, shell ratio, and silk productivity has not been thoroughly investigated. Therefore, there is a need to evaluate fibroin gene expression patterns in diverse bivoltine silkworm strains to identify superior genotypes and generate molecular information that can be utilized in silkworm breeding programmes aimed at enhancing silk yield and quality.

Advances in Fibroin Gene Expression Studies in *Bombyx mori*

Ahmad *et al.* (2004) [1] reported the Immunoblot analysis with proteins isolated from the silk gland of *P. ricini* at different stages of larval development showed that the expression of fibroin heavy chain was developmentally and spatially regulated.

Ahmad *et al.* (2004) [1] revealed significant differences between *P. ricini* and *B. mori* in the biochemical composition and immunochemical characteristics of fibroin heavy chain. These differences might be responsible for the differences seen in the quality of silk produced by these two silkworms.

Annamalai and Jayaprakash (2012) [2] conducted studies on *Chelifer* sp. of order Cheliferoidea to the class Arachnida. The silk gland of chelicerae was dissected out. The samples of the fibroin protein secreted from the silk gland were subjected for physical, chemical and biological analysis. It was found that false scorpion fiber could be dissolved in 6N HCL/50 per cent propionic acid medium. The amino acid composition was recalled the blend of amino acids present in the fibrous protein of spider and *Bombyx mori*.The structural studies suggesting collagenous in nature and a relationship with silk fibers of spider and silk insect.

Buhroo *et al.* (2016) [3] reported that Twelve bivoltine silkworm *Bombyx mori* L. genotypes were evaluated for cocoon associated traits during spring and summer seasons of 2012 and 2013 respectively. The data generated in respect of different traits was pooled separately, analyzed statistically and subjected to multiple trait evaluation indexes. Out of twelve genotypes, six genotypes viz., SKAU-R-1, SKAU-R-6, SKUAST-31, NB4D2, SH6 and SKUAST-28 were shortlisted for spring season and eight genotypes viz., SKAU-R-1, SKAU-R-6, NB4D2, SH6, SKUAST-31, CSR18, DUN6 and DUN22 for summer season. These genotypes scored higher Evaluation index (E. I)values (>50) and were identified as promising genotypes hence recommended for rearing under temperate climatic conditions to push up silk productivity in the temperate region of India.

Datta *et al.* (2001) [5] investigated the fibroin gene expression during the larval developmental stages of the Saturnidae silkworm, *Antheraea mylitta*, Northern blot analysis of larval silk gland total RNA using the fibroin gene as a probe showed that fibroin is expressed in the inter moult stages and repressed during the moulting stages. Western blot analysis of fibroin protein production with anti-fibroin antibody confirmed the differential fibroin expression, in accordance with fibroin mRNA synthesis. Dot blot hybridization of genomic DNA isolated from each larval developmental stage with the labeled fibroin gene showed that at the genomic level, the relative concentration of the fibroin gene was constant throughout the developmental stages.

Fang *et al.* (2015) [6] reported the *Bombyx mori* was domesticated from the Chinese wild silkworm, *Bombyx mandarina*. Wild and domestic silkworms are good models in which to investigate genes related to silk protein synthesis that may be differentially expressed in silk glands, because their silk productions are very different. Here we used the mRNA deep sequencing (RNA-seq) approach to identify the differentially expressed genes (DEGs) in the transcriptomes of the median/posterior silk glands of two domestic and two wild silkworms. The results indicated that about 58% of the

total genes were expressed (reads per kilo bases per million reads (RPKM) ≥ 1) in each silkworm. Comparisons of the domestic and wild silkworm transcriptomes revealed 32 DEGs, of which 16 were up-regulated in the domestic silkworms compared with in the wild silkworms, and the other 16 were up-regulated in the wild silkworms compared with in the domestic silkworms. Quantitative real-time polymerase chain reaction (qPCR) was performed for 15 randomly selected DEGs in domestic versus wild silkworms. The qPCR results were mostly consistent with the expression levels determined from the RNA-seq data.

Gupta *et al*. (2015) [8] studied the golden silk spun by Indian golden silkworm *Antheraea assama*, is regarded for its shimmering golden luster, tenacity and value as biomaterial. This report describes the gene coding for golden silk H-fibroin (AaFhc), its expression, full-length sequence and structurally important motifs discerning the underlying genetic and biochemical factors responsible for its much sought-after properties. The coding region, with biased isocodons, encodes highly repetitious crystalline core, flanked by a pair of 5′ and 3′ non-repetitious ends. *AaFhc*mRNA expression is strictly territorial, confined to the posterior silk gland, encoding a protein of size 230 kDa, which makes homodimers making the elementary structural units of the fibrous core of the golden silk.

Hwang *et al.* (2001) [9] confirmed that the exon 2 of *A. yamamai* fibroin gene was composed of 80 repetitives with each pair composed of one polyalanine motif (PAM) and one non-polyalanine motif (NPAM). The amino acid sequence of NPAM showed a higher hydrophobic pattern than PAM sequence, and thus, *A. yamamai* fibroin showed the characteristic of the regular repetition of a hydrophilic region and a hydrophobic region.

Hwang *et al.* (2001) [9] reported the northern blot analysis showed that *A. yamamai* fibroin gene was distinctively expressed in the posterior silkgland, and this expression pattern was identical to the characteristic expression pattern of *B. mori* fibroin gene. Thus, it was assumed that the expression mechanism of fibroin gene between *A. yamamai* and *B. mori* is evolutionarily shared in terms of time and space. Northern blot analyses confirmed that fibroin gene was actively expressed in the posterior silkgland of the final instar larvae of *A. yamamai*.

Jian-ying*et al.* (2011) reported that the five genes of silk proteins secreted from posterior silk gland including fibrion heavy (H) and light (L) chains, P25, serion I and serion II basically showed obvious up-regulation at V3 which lasted to V5 (first day of fifth instar), while slight down-regulation at W(wandering stage of fifth instar), they further found that the expression of translation related gene including ribosomal protein and translation initiation factors generally remained stable from M4 (fourth moult) to V5 (fifth day of fifth instar), where it shows clear down-regulation at W.

Kimura *et al.* (1985) [11] showed that the expression of the L-chain gene is, like that of the P25 gene, coordinated with the expression of the fibroin gene during late larval development. Thus, the results indicated that the expression of the P25 and L-chain genes is, similar to that of the fibroin gene, controlled at the transcriptional level.

Kobayashi *et al*. (2009) [12] reported the evolutionary conservation of the gene expression mechanism and secretion machinery between *Antheraea* and *Bombyx* fibroins. They introduced the *A. yamamai* fibroin gene into the domesticated silkworm, *B. mori*. The spliced *A. yamamai* fibroin mRNA appeared only in the posterior region of the silk gland of the transgenic silkworm, suggesting that the functions of the fibroin promoter region and the splicing machinery are conserved between these two species.

Krishan and Konig (2011) reported the proteome of silkworm and showed that the expression of proteins such as lipophorin, vitellogenin, storage protein and 30 KDa proteins in the fat body tissues varied with respect to biotic and abiotic factors.

Lokesh *et al.* (2012) [14] reported the wild silkworm, tropical tassar *Antheraea mylitta* drury being mainly culturing in central and eastern parts of india and produces famous tassar silk. This insect species expresses divergent phenotypic characters in response to varying ecological and climatic conditions thus exists as ecoraces. In the present strudy, three ecoraces viz, Daba, Sukinda (Semi-domesticated) and Sarihan (wild) have been selected for the assessment of the variability in silk gland related traits. The parameters of the silk glands studied like comparative length, weight and silk gland to larval body mass index. The result showed significant varations at p<5% in all the parameters among the three ecoraces studied also showed the sexual dimorphism in expression of silk gland features and economic traits.

Long *et al.* (2015) [15] investigate the role of different parts of the fibroin heavy chain (H-chain) in the secretion of fibroin in the silk gland of the silkworm (*Bombyx mori*) *in vivo*, two enhanced green fluorescent protein (EGFP)/H-chain fusion genes with deduced protein sequences containing an identical N-terminal region and different C-terminal regions of the H-chain were introduced into the *B. mori* genome using a piggyBac-mediated germline transformation. The results revealed that only the non-repetitive N terminus of the H-chain is essential for secretion of the H-chain into the posterior silk gland lumen, there results not only provide a theoretical basis for the genetic modification of silk fiber as a functional biomaterial but also are of great significance to establishing a new silk gland bioreactor to mass-produce exogenous proteins in an active form.

Mahmoud (2017) [16] reported the used sodium dodecyl sulphate polyacrylamide gradient gel electrophoresis (SDS-PAGE) to determinethe protein profiles of two mulberry variety leaves: the cultivated *Morus alba* var. Morittiana and the wild one *Morus laevigata* and their effects on haemolymph and silkgland protein patterns in final larval instar of silkworm *Bombyx mori* L. Results showed some differences in the number and abundance of protein bands in the two studied mulberry variety leaves as they were 5 bands in *Morus alba* var. Morittianaranged between (47.27-344.47 kDa), while, were 10 bands in *Morus laevigata* leaves ranged between (17.79- 350.11 kDa).

Mondal *et al*. (2007) [17] reported that silkworm, *B. mori* produces massive amount of silk proteins during the final stage of larval development and are stored in the middle silk gland and they are discharged through the anterior duct and spinneret, at the end of the fifth instar. The authors reported that two kinds of silk proteins have been distinguished as major components of silk cocoons, the first being fibroin, a fibrous protein composed of heavy (H) chain, Light (L) chain and glycoprotein linked by disulfide bonds and the second being sericin a natural macromolecular protein, serving as an adhesive to unite fibroin for making silk cocoons of silkworm, *B. mori*.

Muhammad *et al.* (2015) [18] reported a simple method of separating silk fractions using formic acid. The formic acid treatment partially releases predominately the light chain fragment (soluble fraction) and then the soluble fraction and insoluble fractions can be converted into new materials. The regenerated original (total) silk fibroin and the separated fractions (soluble vs. insoluble) had different molecular weights and showed distinctive pH stabilities against aggregation/precipitation based on particle charging. All silk fractions could be electrospun to give fiber mats with viscosity of the regenerated fractions being the controlling factor for successful electro spinning.

Murali *et al*. (2018) [19] reported that 5 parental CSR breeds of *Bombyxi mori* L. from the germplasm bank of RSRS, Miran Sahib were evaluated regrading phenotypic and biological/economic parameters of CSR breeds. Observations on the different phenotypic and economic traits of silkworm, *Bombyx mori* L. were taken. The perusal of the data reveals that the larval weight varied in the range of 34.94 to 40.86 g whereas, larval duration was observed in the range of 24.00 days to 26.22 days. The larval length recorded as on 6th day 7.76 cm (CSR - 19) to 8.26 cm (CSR - 2).Single cocoon weight 1.49 to 1.70 g. The highest single cocoon weight was CSR - 18 (1.70 g), Single shell weight 0.28 to 0.36 g. Maximum shell weight recorded in CSR - 18 (0.36 g), Shell percentage 18.86 per cent (CSR - 4) to 21.29 per cent (CSR - 19). Maximum yield CSR - 2 (15.23 Kg), was observed significantly superior compared to others.

Obara and Suzuki (1988) [22], reported the activation of fibroin gene transcription starts from the anterior part of PSG at the beginning of the fifth instar and gradually spreads toward the posterior end. Differential expression of the ubiquitin gene and ubiquitination of proteins in the posterior silkgland of *B. mori* throughout larval stages also participated in fibroin synthesis and degeneration. P25 gene, like the fibroin gene, is actively transcribed during intermolt periods and is repressed during the molting stages. It was found that the level of P25 mRNA in PSG changed in parallel with the level of fibroin mRNA.

Qi *et al*. (2017) [23] reported that the biological performance of artificial biomaterials is closely related to their structure characteristics. Cell adhesion, migration, proliferation, and differentiation are all strongly affected by the different scale structures of biomaterials. Silk fibroin (SF), extracted mainly from silkworms, has become a popular biomaterial due to its excellent biocompatibility, exceptional mechanical properties, tunable degradation, ease of processing, and sufficient supply. As a material with excellent process ability, SF can be processed into various forms with different structures, including particulate, fiber, film, and three-dimensional (3D) porous scaffolds.

Raman *et al.* (2007) [24] reported the silk glands of *Bombyx mori* L. have been used as a model system in the production of large amount of silk proteins prior to cocoon spinning. The amount of silk proteins synthesized could be augmented by improving the nutritional status of silkworm larvae. The present study reports the changes in the silk gene expression by supplementing P-soyatose (hydrolyzed soy protein) during the fifth instar *B. mori* male larvae. Western blot analysis of the silk gland protein in the soy protein supplemented group demonstrated higher staining intensity of H and L chains. The rate of protein synthesis was increased in the male larva reared with P-soyatose in comparison to control group. This was indicated by high incorporation of 3H leucine in the posterior silk glands of supplemented larvae reared with P-soyatose. Northern blot analysis unequivocally prove that supplementation of soy protein increases the synthesis of silk fibroin, as it increases the levels of fibroin mRNA, which reflects fortification in the economic traits of *B. mori.*

Shi *et al*. (2019) [25] reported that the silk gland synthesizes and secretes a large amount of protein and stores liquid silk protein at an extremely high concentration. Interestingly, silk proteins and serine protease inhibitors are orderly arranged in the silk gland lumen and cocoon shells. Silk fiber formation and the spinning mechanism have not been fully elucidated. In total, 3121 differentially expressed genes were identified in the seven segments. Genes highly expressed in the middle silk gland (MSG) were mainly involved in unsaturated fatty acid biosynthesis, fatty acid metabolism, apoptosis—fly, and lysosome pathways, whereas genes highly expressed in the posterior silk gland (PSG) were mainly involved in ribosome, proteasome, citrate cycle, and glycolysis/gluconeogenesis pathways.

Shimura *et al*. (1976) [26] reported the investigated fractionation of fibroin prepared from the posterior silk glands of *Bombyx mori* L. and reported that after carboxy methylation of the fibroin, it was fractionated by ammonium sulfate precipitation, Sephadex G-200 gel filtration and DEAE-cellulose column chromatography. The fibroin was composed of at least two protein groups of large molecular size and three or four components of small molecular size. In addition, a mixture of proteins ranging in size from about 25,000 to more than 100,000 daltons with almost the same amino acid compositions. The results of the study indicate major heterogeneity in the molecular size of posterior silk gland fibroin, in addition, the study also suggested that there may be the possibility of repeating sequences with relatively simple amino acid compositions in major peptide chains of fibroin.

Suzuki (1994) [27] reported the summed up silk gene regulation studies carried out in *Bombyx mori*. The study has brought up the importance of understanding the *Bombyx mori* L. body plan in comparison with the body plans of other organisms.

Suzuki and Suzuki (1974) [28] used a chemical titration procedure to provide the first quantitative measurements of fibroin mRNA synthesis at various stages of PSG development. They demonstrated that mRNA was accumulation during successive feeding stages and decreased in mRNA cellular content in the course of molting stages. It was also confirmed that the transcription of the fibroin gene is turned-off during molting stages, and the preexisting fibroin mRNA molecules are completely degraded. Thus, the results of these studies indicated that fibroin gene expression was controlled at the transcriptional level in a precise temporal and spatial manner, the fibroin gene being expressed at the intermolt stages but not at the molting stages, and only in PSG reported that starvation during the last instar blocked the transcription in the silkgland and decreased the silk protein synthesis.

Wang *et al*. (2006) [31] investigated the functional signal peptide of silkworm fibroin heavy chain (Fib-H) and the effect of N- and C-terminal parts of Fib-H on the secretion of Fib-H *in vivo*, N- and C-terminal segments of Fib-H gene were fused with enhanced green fluorescent protein (EGFP) gene. The fused gene which were introduced into silkworm larvae and expressed in silk gland using recombinant

AcMNPV (*Autographa californica*) multiple nuclear polyhedrosis virus) as vector. The fluorescence of EGFP was observed with fluorescence microscope. Fib-H-EGFP fusion proteins extracted from silk gland were analyzed by Western blot. Results showed the two alpha helices within N-terminal 163 amino acid residues and the C-terminal 61 amino acid residues were not necessary for cleavage of signal peptide and secretion of the fusion protein into silk gland.

Xu *et al.* (2009) [32] analyzed the proteins associated with fibroin synthesis in posterior silkgland of *Antheraea pernyi* fifth instar larvae with 2 DE. Approximately 352±10 spots and 327±10 spots were detected on day 1 and 4, respectively and most of the spots ranged from 14 to 90 kDa. According to the spot density, twenty-three spot up-regulated by more than two folds on day 4 were selected for further identification. Search for homologous sequences in NCBI databases identified seven proteins including GSTT and RPL8 involved in the regulation of transcription, translation and cell metabolism.

Xu *et al.* (2009) [32] investigated the up regulated proteins associated with fibroin synthesis in posterior silk glands of fifth instar of *A. pernyi.* Real-time quantitative PCR showed that the expression of glutathione S-transferase theta (GSTT) peaked on day 4, whereas the ribosomal protein L8 (RPL8) peaked on day 3, confirming the up-regulation of these proteins during last larval instar and these results showed that expressions of GSTT and RPL8 may play a role in fibroin synthesis in *A. pernyi.*

Yamaguchi *et al.* (1989) [33] determined the primary structure of Fib-L of *B. mori* J-139 by nt sequencing of Fib-L eDNA clones, pFL18 and pFL96, and by aa sequence analysis of Fib-L peptides. It was found to consist of seven exons spread within a genomic DNA fragment 13472 bp in length. The length of mature L-chain mRNA was about 1.2kb. The gene contained a large (8145bp long) first intron accounted about 60 per cent of the gene. In the 5' flanking region there was a TATA-sequence (5'-TATATAAAT-Y between nucleotides -33 and -25) and a 5'-GTCAATTT-3' sequence (-128 to -121), that might serve as promoters for transcription of the L-chain gene. A high degree of homology between 5' flanking regions of the L-chain gene and fibroin gene was reported; this particularly concerns regions -416 to -227 in L-chain gene and -273 to -193 in fibroin gene.

Yan *et al.* (2003) [34] analyzed the changes in protein expression patterns of the posterior silk gland of fifth instar from the p50 silkworm strain. The study found that individual silkworms expressed proteins consistently regardless of the part of the posterior silk gland used.

Yang *et al.* (2010) [35] determined the protein expression profiles in the fat bodies of larval, pupal and moth stages of silkworm using shotgun proteomics and MS sequencing. They identified 138, 217, and 86 proteins from the larval, pupal and moth stages, respectively, of which 12 were shared by the 3 stages. There were 92, 150, and 45 specific proteins identified in the larval, pupal and moth stages, respectively. Among the specific proteins identified in moth fat body, sex-specific storage-protein 1 precursor and chorion protein B8 were unique to the moth stage, indicating that the moth stage fat body is more important for adult sexual characteristics.

Zhao *et al.* (2015) [36] reported that the genes responsible for silk biosynthesis are switched on and off at particular times in the silk glands of *Bombyx mori*. This switch appears to be under the control of endogenous and exogenous hormones. However, the molecular mechanisms by which silk protein synthesis is regulated by the juvenile hormone is largely unknown. Far-Western blots, enzyme-linked immune sorbent assays, and co-immuno precipitation assays revealed that Bmdimm protein interacted with another basic helix-loophelix transcription factor, Bmsage. Immuno staining revealed that Bmdimmand Bmsage proteins are co-localized in nuclei. *Bmdimm* expression was induced in larval silk glands *in vivo*, in silk glands cultured *in vitro*, and in *B. mori* cell lines after treatment with a JH analog.

Zhou *et al.* (2000) [37] reported the H-chain is a fibrous protein of 5263 residues and characteristically rich in glycine (46%), alanine (30%) and serine (12%) amino acids. The bulk of the H-chain polypeptide consisted of a low-complexity region (4754 residues) and was made up of 12 GX dipeptide repeat motifs (X is Ala in 65%, Ser in 23% and Tyr in 10%) separated by 11nearly identical boundary sequences It has been suggested by a wide array of authors that the glycine-rich domains and the (Gly-Ala) n repeat motifs may have a bearing on the quality of silk produced by mulberry silkworms.

Zhou *et al.* (2008) [38] reported the proteomic profiles of midgut from silkworms fed on different diets were compared to determine the effects of differential nutrient conditions on its digestion and absorption .The results indicated that the expression of Profilin, Serpin-2 and Glutathione peroxidase were up-regulated in midgut of silkworm fed on artificial diet, while Myosin 1 light chain and Tropomyosin were down-regulated.

Conclusion

Fibroin gene expression plays a fundamental role in determining silk production and quality in *Bombyx mori*. The extensive research conducted over the past decades has demonstrated that fibroin synthesis is a highly regulated biological process controlled at transcriptional, translational, and post-translational levels within the posterior silk gland. Variations in the expression patterns of fibroin-related genes among different silkworm strains contribute significantly to differences in cocoon yield, shell weight, filament characteristics, and overall silk productivity. Recent advances in molecular biology, transcriptomics, and proteomics have enhanced our understanding of the genetic mechanisms governing fibroin biosynthesis and secretion. These studies have identified key regulatory pathways, developmental stages, and environmental factors influencing fibroin gene expression. The accumulated evidence suggests that expression profiling of fibroin genes can serve as a reliable molecular tool for assessing genetic diversity and identifying elite silkworm strains with superior silk-producing ability. Therefore, integrating fibroin gene expression studies with conventional breeding programmes can accelerate the development of high-yielding and quality silk-producing bivoltine hybrids. Future research should focus on functional genomics, gene regulation networks, and marker-assisted selection to exploit fibroin-associated genetic variation more effectively. Such approaches will contribute substantially to sustainable sericulture development and increased silk productivity under diverse agro-climatic conditions.

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