International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 19-12-2021, Accepted: 04-01-2022, Published: 20-01-2022
Volume 7, Issue 1, 2022, Page No. 73-80

Transgenesis in silkworm: An overview

Arti Sharma[1*], R K Gupta[2], Kapil Attri[4], Sumiya Afreen[4], R S Bandral[2], Kamlesh Bali[3]
[1] Research Scholar, Division of Sericulture, SKUAST-Jammu, Jammu and Kashmir, India
[2] Professor, Division of Sericulture, SKUAST-Jammu, Jammu and Kashmir, India
[3] Associate Professor, Division of Sericulture, SKUAST-Jammu, Jammu and Kashmir, India
[4] Division of Sericulture, SKUAST-Jammu, Jammu and Kashmir, India

Abstract
Transgenic technology is the science of intentionally introduction of a foreign gene or genetic construct into the genome of a target animal. The transgenic technology in silkworm provides a new system for the enhancement of anti-viral capacity, production of recombinant human proteins including antibodies and membrane proteins, silkworm strains resistant to *Bm*NPV, production of biomaterials and high toughness silk, development of drugs and high yielding silkworm strains and biomedical applications as drug carriers, sensors or scaffolds for tissue engineering. In addition to this, the transgenic silkworms are also developed to use as a novel animal model for testing medicines based on metabolic similarities between silkworms and mammals. Silkworm has been proven to be one of the most proficient and popularly used eukaryotic expression tools. The silkworm has turn out to be a perfect multicellular eukaryotic model system for basic research.

Keywords: silkworm, transgenesis, biomaterial, silk, genetic engineering

Introduction
The advent of genetic engineering has enabled us to transform an organism's genetic makeup in a variety of novel ways. One of them is transgenic technology. Transgenic technology is the science of intentionally introduction of a foreign gene or genetic construct into the genome of a target animal. A transgenic or genetically modified organism can be defined as an animal in which there has been a deliberate modification of its genome, the genetic makeup of an organism responsible for inherited characteristics, through a human technological intervention. Transfer of exogenous genes into the genome is an important technology in modern life sciences and is one of the most significant advances in experimental and applied biology in the past 21 years. Transgenic animals are broadly used to learn *in vivo* gene purpose all through growth, organogenesis and aging as well as to model human diseases. Animal transgenic technology is worn to incorporate distant genes into the animal genome by genetic engineering technology so that distant genes can be articulated and inborn to the progeny. The competence of the transgenesis procedure and accurate control of gene expression are the key limiting factors on preparation of transgenic animals. With the in depth research, the transgenic technology will have broad application prospects in the fields of exploration of gene function, genetic improvement of animals, and production of pharmaceutically valuable proteins using animal bioreactor, animal disease models, and organ transplantation and so on. The silkworm, *Bombyx mori* L has become a useful model of the Lepidoptera, with an increasingly important role in basic biological research. Completion of the framework of the *Bombyx mori* genomic map in 2004 marked the start of the era of *Bombyx mori* functional genomics and transgenic technology, which has received widespread attention.

Transgenesis in Silkworm
Drosophila melanogaster was the first insect species in which transgenesis (insertion of a foreign gene into the genome) was accomplished by Rubin and Spradling in 1982 [21]. Numerous attempts to use this method in other insects failed because the P element transposon could not be mobilized and inserted into the genome of the non-drosophilid species. This became possible only after the discovery of transposons whose activity was not restricted to *Drosophila* species. The earliest triumphant transgenesis of a non-drosophilid creature was reported for the medfly *Ceratitis capitata* injected with the transposon vector *Minos* (Loukeris *et al*., 1995) [18]. A transgenic silkworm *Bombyx mori* was first produced by gene transfer utilizing a *piggyBac* transposon-derived vector by Tamura *et al*. in 2000 [26]. Since then, transgenic silkworms have been developed to produce recombinant human proteins, including procollagen, cytokines and monoclonal antibodies in their cocoons and silk glands. The advantages of transgenic silkworms producing recombinant human proteins are: somewhat larger amounts of recombinant proteins (> 1 mg per larva and/or cocoon) are expressed than those derived from other hosts, physical containment of transgenic silkworms is easy because the larvae move slowly and seldom escape and because the moths cannot fly, human pathogens have not been found in *Bombyx mori* and year round breeding is possible with an artificial diet and expanded facilities, reducing the cost of producing recombinant proteins. Silkworm larvae have a lot of advantages as a biofactory since they can be effortlessly reared using mulberry leaves on large scale at much lower expenditure or artificial di*et al*l through the year, their bodies are large and easy to operate, they have a comparatively short life cycle (approximately 7 weeks), their genetics and biology have been well documented and it is easy and safe for management. It is very promising to use the silkworm as a bioreactor for large scale industrial mass production.

Table 1: Significant studies pertain to Silkworm Transgenesis from 2000 to 2020.

Reported Outcome	Author	Year
Developed a system for stable germline transformation in the silkworm, *Bombyx mori* L. using a *piggyBac* transposon-derived vector discovered in lepidopteran *Trichoplusia ni*	Tamura *et al.*	2000
Evaluated a 3×P_3 – EGFP marker that facilitates screening for transgenic silkworm *Bombyx mori* L. from the embryonic stage onwards	Thomas *et al.*	2002
Generation of transgenic silkworms that produce cocoons containing recombinant human collagen	Tomita *et al.*	2003
Used Electroporation as a methodology to introduce foreign genes into silkworm eggs and found that the ratio of foreign gene entering is voltage dependent. The *in vitro* transcribed transposase mRNA facilitated transposition to take place earlier.	Guo *et al.*	2004
Generated hybrid transgenic silkworms whose PSGs are capable of producing mini-collagens and enough P4H for their prolyl 4-hydroxylation. The P4H activity in the transgenic silkworms was 130 fold higher than that of wild-type counterparts.	Adachi *et al.*	2006
The transformation efficiency of the *piggyBac* based system might vary with silkworm strains with different genetic backgrounds.	Zhong *et al.*	2007
Constructed H-chain expression system to produce recombinant proteins in the cocoon of transgenic silkworms.	Kurihara *et al.*	2007
Developed an efficient jumpstarter strains by inserting the *piggyBac* transposase gene under the control of *Bombyx* Cytoplasmic actin gene (*Bm*A3) promoter into the genome, to construct an enhancer trap system in the silkworm, *Bombyx mori* L.	Uchino *et al.*	2008
Due to transgenic breeding of anti-*Bombyx mori* L. nuclear polyhedrosis virus silkworm, *Bombyx mori* the morbidity ratio of the nuclear polyhedrosis decreased from 90% in the original silkworm strain to 66.7% in the transgenic silkworm strain.	Yang *et al.*	2008
Transgenic silkworms expressed significant amounts of the μ-opioid receptor in the fat body and the silk gland and exhibited ligand affinity similar to that of an authentic sample.	Tateno *et al.*	2009
Generated germline-transgenic silkworms that spun cocoons containing recombinant spider silk by piggyBac-based transformation vector. Compared with wild-type silk, the recombinant silk displayed a higher tensile strength and elasticity.	Wen *et al.*	2010
On the W chromosome of silkworm, *Bombyx mori* L. found a novel piggyBac like DNA transposon that encodes an intact transposase, flanked by 16-bp perfect inverted terminal repeats and a duplicated TTAA target site.	Daimon *et al.*	2010
Transgenic silkworms encoding chimeric silkworm/spider silk proteins on average tougher than the parental silkworm silk fibers and as tough as native dragline spider silk fibers.	Teule *et al.*	2012
Germline transformation by sperm mediated gene transfer in silkworm confirmed the positive rate of transgenesis.	Zhou *et al.*	2012
A transgenic *Bm*N cell line of *piggyBac* transposon-derived targeting expression of humanized proteins established to produce “bisected” complex N-glycans like in mammalian cells.	Hu *et al.*	2012
Efficient system for producing recombinant proteins was constructed using a transgenic silkworm and suitable for the production of recombinant silk as a fiber for making fabrics and biomaterials for medical purposes.	Tatemastu *et al.*	2012
In transgenic silkworms the bHsp70 promoter can be used for the rapid and simple screening.	Kim *et al.*	2013
Expression system for production of bioactive compounds, recombinant human adiponectin, in the silk glands of transgenic silkworms	Shin *et al.*	2014
In transgenic silkworms the tensile characteristics of the raw silk improved by 53% after the introduction of spider dragline silk proteins.	Kuwana *et al.*	2014
In silkworms the anti-viral capacity can be improved by transgenic technology such as overexpression of an endogenous or exogenous antiviral gene, RNA interference of the *Bm*NPV gene or regulation of the immune pathway to inhibit *Bm*NPV at different stages of infection.	Jiang and Xia	2014
Advanced technologies for genetically manipulating the silkworm, *Bombyx mori*, a model lepidopteran insect and their applications to the study of gene function and their use in genetically modifying *Bombyx mori* for biotechnology applications.	Xu Hanfu and O’Brochta David A.	2015
Developed an efficient strategy that combines a method for the post-integration elimination of all transposon sequences, a site-specific recombination system, and an optimized fibroin H-chain expression system to produce a stable, replaceable, highly efficient transgene expression system in silkworm, *Bombyx mori* that overcomes the disadvantages of random insertion and post-integration instability of strains.	Long *et al.*	2015
Established a transgenic silkworm stably expressing a human-mouse chimeric anti-CD20 mAb having the same amino acid sequence as rituximab and results showed a similar antigen-binding property, but stronger antibody-dependent cell-mediated cytotoxicity (ADCC) and weaker complement-dependent cytotoxicity (CDC) compared to MabThera.	Tada *et al.*	2015
Explored a strategy for the mass production of r-haFGF protein with biological activity in the transgenic silkworm cocoons.	Wang *et al.*	2015
Transgenic silk fibroin had relatively higher Ca-binding activity than unmodified silk fibroin.	Wang *et al.*	2016
Transgenic silkworms overexpressing human lysosomal enzymes in the silk glands could serve as future bio-resources that provide safe therapeutic enzymes for the treatment of Lysosomal storage diseases (LSDs).	Itoh *et al.*	2016
Transgenic silkworm lines overexpress Cecropin B or Moricin antimicrobial peptides at the level of silk gland and characterized by increased antimicrobial properties to inhibit the bacterial growth.	Saviane *et al.*	2018
Successfully replaced the 16 kb endogenous *Fib*H gene with a 1.6-kb *Ma*Sp1 gene fused with a 1.1-kb partial *Fib*H sequence and achieved up to 35.2% chimeric *Ma*Sp1 protein significant changed the silk fiber in transgenic silkworm.	Xu *et al.*	2018
An efficient strategy to produce PDGF-BB in large quantities using a transgenic silkworm was obtained. A protein quantity of approximately 0.33 mg/g was found in the cocoon, with a purity of 82%.	Chen *et al.*	2018
Produced transgenic silkworms which overexpress human lysosomal enzymes in silk glands, and have purified active and functional enzymes form middle silk glands and cocoons.	Itoh *et al.*	2018

Feeding Calcium lignosulfonate alone improved the mechanical properties of silk in transgenic silkworm.	Zhang *et al.*	2019
Established a silkworm based silk gland bioreactor for high-efficiency production of recombinant human lactoferrin with antibacterial and anti-inflammatory activities	Xu *et al.*	2019
*Bm*GT1-L ectopic expression in the transgenic silkworm posterior silk gland promoted glycine biosynthesis and enhanced silk yield via increasing fibroin synthesis.	Tang *et al.*	2020

Applications of Silkworm Transgenesis

Enhancement of Anti-viral capacity in silkworm

The Silkworm, *Bombyx mori* L. faces biological challenges from various pathogens including viruses, fungi and bacteria, which cause losses of almost 20% of cocoon crop production each year. About 80% of total cocoon loss is due to the occurrence of viral diseases. These diseases are caused by *Bombyx mori* Nucleopolyhedrovirus (*Bm*NPV), *Bombyx mori* cytoplasmic polyhedrovirus (*Bm*CPV) or *Bombyx mori* densovirus (*Bm*DNV). Of these diseases, *Bm*NPV is the most widespread risk to sericulture in almost all countries (Yang *et al.*, 2008) [44]. Resistant strains of silkworms can be obtained by traditional or transgenic approaches. The two mainly significant characters in breeding silkworm strains are disease resistance and economic characteristics. Conventional breeding methods have restrictions such as enhancing pathogen confrontation at the cost of the quality of economically important characteristics. The restrictions of conventional breeding methods can be avoided by the use of transgenic technology, which theoretically changes simply the target trait. The antiviral capacity of the silkworms can be improved by transgenic technology such as over expression of an endogenous or exogenous antiviral gene, RNA interference of the *Bm*NPV gene, or by the regulation of the immune pathway of the organism to inhibit *Bm*NPV at different stages of infection. Over expression and RNA interference (RNAi) are two established gene regulation strategies that have been applied in some organisms to improve pathogen resistance (Jiang and Xia, 2014) [12]. Improvement of antiviral competence by transgenic technology in the silkworm has important theoretical and practical values and could encourage antiviral research in other animals breeding also.

Production of recombinant human lactoferrin protein

Human lactoferrin is an iron-binding glycoprotein that is extensively found in exocrine secretions counting, milk, tears, saliva, bile, serum, gastrointestinal fluids, and vaginal fluids with large quantity. Human lactoferrin has various biological functions, in particular, widely known bactericidal and bacteriostatic activities that form the primary line of defense against the microbial infections and in general, antitumor, antioxidant and stimulate the proliferation in many cell types. With the growth of genetic manipulation tools, in particular the successful establishment of the *piggyBac* transposon-mediated genetic transformation technology in silkworms, it is achievable to genetically engineer the silkworm silk gland as an ultimate bioreactor for the recombinant expression of additional precious foreign proteins all along with production of their silk proteins. Till now more than ten recombinant proteins with various bio-functions and application potential have been successfully expressed in the silk glands of transgenic silkworms and cocoons, including human collagen (Adachi *et al.*, 2006) [1], human serum albumin, antibodies (Kurihara *et al.*, 2007), recombinant human μ-Opioid receptor (Tateno *et al.*, 2009) and human growth factors (Wang *et al.*, 2015)[].

Fig 1: Applications of Silkworm Transgenesis

Production of silkworm strains resistant to *Bm*NPV

Nuclear polyhedrosis is caused by the *Bombyx mori* nuclear polyhedrosis virus (*Bm*NPV), a member of the subfamily *Eubaculovirinae* of the family *Baculoviridae*, commonly known as Grasserie. It is one of the deadliest diseases that can strike the mulberry silkworm, *Bombyx mori*. The most economical and effective way to prevent the disease is by breeding the antivirotic silkworm strains. Previous genetic development research has shown that resistance to *Bm*NPV is controlled by one major gene and several minor genes belonging to the incomplete patroclinous heredity with the effective gene number of 2.31. Silkworm strains resistant to *Bm*NPV virus were obtained by transgenic technology experiments in silkworm. To produce a silkworm strain resistant to *Bm*NPV, a *piggyBac* transposon with an A3 promoter were indiscriminately inserted into the silkworm along with the enhanced green fluorescent protein (EGFP) reporter gene into the silkworm genome. PCR results verified the insertion of the extraneous EGFP gene, and fluorescence microscopy showed that the EGFP was expressed in the midgut tissue. The morbidity ratio of the nuclear polyhedrosis decreased from 90% in the original silkworm strain to 66.7% in the transgenic silkworm strain. Compared with the resistance to the other strain which is commonly used in the production, there was an increase of 33 centesimal points in the transgenic silkworms (Yang *et al.,* 2008) [44].

Production of biomaterials using transgenic silkworms

The silkworm, *Bombyx mori* L. produces silk which is a natural protein fiber that consists of two main components: fibroin and sericin. Silk fibroin has advanced mechanical properties such as the capability to be modified, time-consuming degradation, sufficient time allowed for remodeling and most significantly biocompatibility owing to these properties it becomes perfect biomaterial for clinical uses. The suture prepared from the silk fibroin has been used in pharmaceutical industry for decades. Silk fibroin derived sponge has been used as a scaffold for chondrocyte distribution and cartilage rejuvenation. Due to production of calcium binding silk scaffolds using transgenic silkworms with Ca-binding sequence bone repair has been explored. After the examination of the Ca-binding activity and mineralization of the transgenic silk fibroin *in vivo* it was reported that the transgenic silk fibroin had relatively higher Ca-binding activity than unmodified silk fibroin (Wang *et al.,* 2016) [16]. The amplified Ca-binding activity could encourage the usage of silk fibroin as a biomaterial in the pharmaceutical manufacturing.

Production of high toughness silk by transgenic silkworm

A number of orb web spiders, such as *Nephila* and *Araneus* genera create a variety of silks that have exceptional mechanical properties. Dragline silks are among the strongest fibers, about threefold tougher than aramid fibers and fivefold stronger than steel. Many studies have investigated the manufacture of artificial spider silks in a variety of organisms. Though, long fibers can be obtained merely from silkworms. The silkworm, *Bombyx mori*, silk is composed of fibrous fibroin contained within a sericin protective coating. The fibroin consists of three proteins namely fibroin heavy chain (H-chain), fibroin light chain (L-chain) and fibrohexamerin protein (fhx/P25). The H-chain of the fibroin is supposed to be linked with the mechanical properties of silk. In 2007, the transgenic silkworm in which the spider dragline protein gene cloned was generated, that expressed the fusion protein of the fibroin heavy chain and spider dragline protein in cocoon silk was reported. In the transgenic silkworms, the tensile characteristics of the raw silk improved by 53% after the introduction of spider dragline silk protein (Kuwana *et al.,* 2014) [16]. The silk fibres produced by these animals were composite materials that induced chimeric silkworm/spider silk proteins integrated in an extremely stable manner and are on average tougher than the non-transgenic silkworm silk fibers and as tough as native dragline spider silk fibers. It demonstrates that silkworms can be engineered to manufacture composite silk fibers containing stably integrated spider silk protein sequences, which significantly improves the overall mechanical properties of the parental silkworm silk fibers.

Production of drugs and glycoproteins by transgenic silkworms

Transgenic silkworms have been developed to manufacture recombinant proteins with therapeutic potential for future clinical use, including antibody preparations. Transgenic silkworms overexpressing human lysosomal enzymes in the silk glands are produced and catalytically active enzymes from the middle silk glands have been purified (Itoh *et al.,* 2018) [11]. Numerous biomedically significant proteins, including antibodies, cytokines, anticoagulants, blood clotting factors, etc are glycoproteins and have been implicated in a wide range of important biochemical and biological functions, counting protein steadiness, immune purpose, enzymatic role, cellular linkage and others. There is a high demand for systems that can be used to produce recombinant glycoproteins for basic research and clinical applications, and this objective can be fulfilled with the use of transgenic silkworm strains. The silkworm, *Bombyx mori* is an ideal bio-factory to produce the exogenous proteins through two manners, baculovirus expression system (BES) and transgenesis (Hu *et al.,* 2012) [7].

Enhanced silk yield by transgenic silkworm

The silkworm *Bombyx mori* L. is a lepidopteran economic insect producing plentiful silk fiber in the silk gland. The silk gland is divided into three parts i.e. anterior, middle and posterior regions. The middle silk gland produces sericin and the posterior silk gland secretes fibroin. Fibroin synthesis in the posterior silk gland plays key roles in the silk yield. Moreover, glycine, alanine and serine are the main amino acids in fibroin protein and the three amino acid residues account for 45.9, 30.3 and 12.1 % respectively. This indicates that glycine, alanine and serine are important raw materials in the process of fibroin synthesis. Research has identified 354 candidate genes that are vital during the domestication process. Some of them are highly expressed in the silk gland or midgut and have the potential to enhance silk yield. Interestingly, *Bm*GT1-L belongs to the above mentioned genes and it is also a midgut enriched gene. It was reported that *Bm*GT1-L regulated the glycine-serine biosynthetic pathway to affect cross-talk about nutritional cues between tissues. In addition, ectopic expression of *Bm*GR1-L in the silk gland of a transgenic silkworm improved the silk yield (Tang *et al.,* 2020) [27].

Production of silk with antimicrobial properties

Silk is a high value but low volume product accounting for only 0.2% of world's total textile production. In order to widen the use of silk fabrics and silk based materials, different strategies to obtain silk with antimicrobial properties have been attempted. The use of silver, either as nanoparticles or by means of novel electro less plating technologies is one of the most popular approaches to confer antimicrobial activity on silk as well as on other textiles. However, the transgenic toolboxes available for silkworm allow the development of alternative cost effective strategies, based on the generation of silkworm lines producing cocoons where AMPs are integrated among the silk proteins. The use of biological agents such as antimicrobial peptides (AMPs) is a modern worth-noting trend in the framework of antimicrobial treatments (Saviane *et al.,* 2018) [22]. AMPs are the foremost effectors of the innate immune response and include a heterogeneous group of small peptides. Mature AMPs exert their antimicrobial activity against a wide range of pathogens. To this intend, silkworm-based transgenic techniques come into view to be cost-effective strategies to achieve cocoons in which antimicrobial peptides are integrated among the silk proteins.

In recent times, cocoons transgenic for a recombinant silk protein conjugated to the silkworm Cecropin B antimicrobial peptide were obtained and showed enhanced antibacterial properties.

Table 2: List of protein production in the cocoon of transgenic silkworm

S. No.	Desired Gene	Silk Gland Region	Yield	Application	Source
1.	Partial collagen sequence	PSG	8.4 µg/mg of cocoon	Protein and biomaterial	Tomita *et al.* (2003)
2.	Fibrohexamerin	PSG	0.13 µg/mg	Model protein	Royer *et al.* (2005)
3.	Collagen sequence fused to fibroin	PSG	Not disclosed	Biomaterial	Adachi *et al.* (2006)
4.	bFGF fused to fibroin	PSG	0.4 µg/mg	Biomaterial	Hino *et al.* (2006)
5.	Human serum albumin	MSG	3.0 µg/mg	Therapeutics	Ogawa *et al.* (2007)
6.	Feline interferon	PSG	10-60 µg/mg	Therapeutics	Kurihara *et al.* (2007)
7.	EGFP	MSG	7.0 µg/mg	Model protein	Tomita *et al.* (2007)
8.	EGFP fused to fibroin	PSG	9.5-14.5 %	Model protein	Shimizu *et al.* (2007)
9.	Fibeonectine fused to fibroin	PSG	2-4 µg/mg	Biomaterial	Yanagisawa *et al.* (2007)
10.	EGFP fused to fibroin h-chain	PSG	24 µg/mg	Model protein	Kojima *et al.* (2007)
11.	EGFP with polyhedron	MSG	Not disclosed	Model protein	Iizuka *et al.* (2008)
12.	Mouse monoclonal antibody	MSG	11 µg/mg	Diagnostics & therapeutics	Iizuka *et al.* (2009)
13.	EGFP fused to fibroin	PSG	131-170 µg/mg	Model protein	Zhao *et al.* (2010)
14.	Spider silk fibroin	PSG	Not disclosed	Biomaterial	Zhu *et al.* (2010)
15.	Human collagen	MSG	80 µg/mg	Biomaterial, DDS & therapeutics	Adachi *et al.* (2010)
16.	Soluble GM-CSF	MSG	Not disclosed	Diagnostics	Urano *et al.* (2010)

Production of human growth factors in cocoons of transgenic silkworms

Human platelet derived growth factor (PDGF) belongs to the glycoprotein dimer family and plays an vital role in many processes, such as cell proliferation and wound healing, owing to its strong action as a mitogen for a variety of cell types, especially vascular endothelial cells (VECs) and bone marrow mesenchymal stem cell. PDGF is regarded as a desirable pharmaceutical and recently approved by the FDA for osteochondritis (OCT) and cardiovascular disorder treatment in the clinical setting. With the help of transgenic technology, the production of PDGF-BB in large quantities could be obtained. The high transcriptional expression of the PDGF-BB gene in the transgenic silkworm competitively inhibited the transcription expression of the endogenous sericin-1 gene which caused a significant 37.5% decline. A protein quantity of approximately 0.33mg/g can be obtained in a cocoon. Following a purification process, approximately 150.7µg of recombinant PDGF-BB with a purity of 82% can be obtained from 1 g of cocoons (Chen *et al.*, 2018) [3]. The bioactivity assays showed that the purified recombinant PDGF-BB able to promote the growth, proliferation and migration of BIH/3T3 cells significantly. Thus, the silk gland bioreactor can produce active recombinant PDGF-BB as an efficient mitogen and wound healing agent.

For the creation of large scale bioactive recombinant human acidic fibroblast growth factor in transgenic silkworm, the sequence optimized haFGF was inserted into an enhanced sericin-1 expression system. In result, the expression of r-haFGF protein in the mutant line achieved a 5.6 gold increase compared to the original strain. In the transgenic silkworm strain the high content of r-haFGF facilitated its purification and large scale yields. In addition, the r-haFGF protein bioactively promoted the growth, proliferation and migration of NIH/3T3 cells, signifying the r-haFGF protein possessed native mitogenic activity and the potential for wound healing (Wang *et al.*, 2015) [37].

Recombinant human type III procollagen production by transgenic silkworm

Due to its strength and stability as well as its general compatibility with living cells collagen is having many medical applications, like tissue engineering and drug delivery materials. Currently, the main source of collagen is cow skin and contamination in this source carries a high risk and can also cause reactions. So, it is necessary to find alternative sources of collagen to produce it in large quantities. The domesticated silkworm synthesizes large amounts of silk protein in its silk glands and spins it into cocoons during the fifth larval instar. Recently, a stable germline transformation method in *Bombyx mori* was developed using a *piggyBac* transposon–derived vector. Silkworm is therefore a good candidate host for the production of foreign proteins at an industrial scale. The complementary DNA of type III collagen is an appropriate choice for a transgene because of its simple gene composition. Procollagen mini-chain originally designed by

Lees and Bulleid which is composed of an N-propeptide, one fifth of a triple-helix domain, and a C-propeptide. The *Bombyx mori* synthesized the fusion protein in silk glands and secreted it into cocoons. The fusion proteins were purified to a single band on electrophoretic gels. It is possible to produce approximately 4.5 kg of collagen per year in a facility with a floor surface of about 350 m^2 and five workers caring for a total of about one fifty thousand silkworms and these worms produce a total of about 600 kg of cocoon material, which translates into the predicted 5 kg of total collagen production (Tomita *et al*., 2002) [32].

Conclusion

The development of pharmaceutical and medical applications by means of the domesticated silkworm, *Bombyx mori* L, was recognized in 2000 by using innovative transgenic technology. The transgenic technology provides a new system for the enhancement of anti-viral capacity in silkworm, production of recombinant human proteins including antibodies and membrane proteins, production of silkworm strains resistant to *Bm*NPV, production of biomaterials and high toughness silk, development of drugs and high yielding silkworm strains etc. The recombinant protein production system in silkworm is currently competent of producing a maximum of about 15 mg recombinant protein per silkworm larva. A number of these recombinant proteins have been in marketable use since 2011. In addition to this, the transgenic silkworms are also developed to use as a novel animal model for testing medicines based on metabolic similarities between silkworms and mammals. Remarkable achievements have been attained during the last decade in the field of transgenic technology especially in the development of gene transfer techniques. Novel techniques includes germline stem cell mediated transfer, gene targeting, RNA interference (RNAi)-mediated gene silencing technology, sperm mediated gene transfer etc. These new transgenic techniques were aimed at increasing accuracy and efficiency of the transgenesis so that they can provide a better platform for the study of transgenic animals and encourage the development of medical sciences, livestock production and other fields. Transgenic technology had revolutionized the production of biopharmaceuticals products with efficiencies far superior than any conventional microbial or cell culture production systems.

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