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# Advances and applications of bioinformatics in Sericultural research

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**Abstract**
Seri bioinformatics tools and techniques not only facilitated detection of proteomic and genomic diversity among the species/strains, but also resulted in finding a gap in the silkworm genome sequence of a strain that diverged during the course of domestication. Seri-bioinformatics databases are a valuable seri-bioresource. The available online resources on silkworm and its related organisms, including databases as well as informative websites help to make silkworms healthier, more disease resistant and more productive. These databases provides information on gene, protein sequences and diseases and play crucial roles in conservation of the silkworm species and mulberry plants (Singh *et al*., 216). Bioinformatics approaches give an insight, uncovering the lineage with gene and protein count of *B. mori* and Drosophila encompass ~18,000 and ~16,000 (Genes) and ~9,000 and ~22,000 (Proteins) respectively (Somshekar and Borgowda, 2013).

**Keywords:** database, genomic, mulberry, proteomic and Seri-bioinformatics

## Introduction
The Bioinformatics centre at Central Sericultural Research and Training Institute (CSRTI), Mysore was established as a sub node of the BTISnet in 1999 primarily to support the biotechnological research in sericulture. Maintain information repository of silkworm and mulberry genotypes and breeds. Develop and maintain the databases related to mulberry and silkworm genomes with structural information. To conduct training/workshops to create awareness and in sight in the field of Bioinformatics in general and Seri-bioinformatics in particular. To provide online support information in the field of sericulture Design of online course packages in bioinformatics and allied areas. Maintain the center's website and host the databases and other vital information over the website of the centre.

## MulDis
A Comprehensive Mulberry Disease and Pest Database
MulDis an organism specific database featuring the information on the diseases and pests of the host plant mulberry and the silkworm. The Database provides detailed information on the diseases and pests, place, mode of infection, biotic and abiotic factors, and the cost-effective and eco-friendly strategies adopted for their effective management

## SilkDis
A Comprehensive Silkworm Disease and Pest Database
Organism specific database featuring the information on the diseases and pests of the host plant mulberry and the silkworm. The Database provides detailed information on the diseases and pests, place, mode of infection, biotic and abiotic factors, and the cost-effective and eco-friendly strategies adopted for their effective management.

## BioinfoLib
Under this Bioinformatics Library information system, one can access specialized bioinformatics centers as well as books, scientific journals, manuals, newsletters and the information on latest research & development in those institutes on bioinformatics and allied areas. This library will be accessible over Internet through the URL of the centre that can be used by all the scientists/research students and the teachers of the research institutes and universities

## Silkprot
An Annotated Protein database for Silkworm
SilkProt is a comprehensive, fully annotated, organism specific database for silkworm proteins. The database structure will support users to perform a BLAST search with highly specified and updated latest version of NCBI-BLAST server. In future this database will be extended for structure visualization and pathway information of silkworm proteins.

## Soilinfo
This database provides information about the different types of soils, physical information, physico-chemical properties, primary and secondary micronutrients etc.
Construction of growth indices in popular breeds/hybrids of silkworm *Bombyx mori* L. in relation to nutritional conditions
A software for estimating the Growth Index in silkworm *Bombyx mori* has been developed to compare the growth rate of different silkworm breeds and hybrids under varying conditions of nutrition.

## Database of DNA sequences for important plant genes in mulberry
A database has been developed for storing and retrieving

DNA sequences of important plant genes responsible for yield, quality, diseases and pest resistance and also some of the mulberry specific genes obtained from the public domain.

**SilkPPI- Silkworm protein-protein interaction database**

The Silkworm, *Bombyx mori* protein-protein interaction network were predicted using the well-recognized Interlog method.

**Mulberry Genome Database**

Phylogenetic relationship in terms of dendrogram and marker segregation pattern has been compiled and stored using relational database with Visual Basic Platform. The database is also made available in the form of CD.

**SilkTF-Silkworm Transcription Factor Database**

Transcription factors are the key regulatory proteins that enhance or repress the transcriptional rates. Regulation of gene expression. Identification and classification of TFs provide important resources for researchers especially in the comparative genomics and transcriptional regulation.

**Table 1:** Databases and their URL for quick access to information on mulberry, silkworm and other allied field.

| |
|---|
| http://www.ab.a.u-tokyo.ac.jp/silkbase/ http://www.ag.auburn.edu/enpl/hyche/saturniidae/ http://amigo.geneontology.org/cgi-bin/amigo/ blast.cgi www.arthropodgenomes.org/wiki/i5K http://www.bioinformaticsonline.org http://www.butterflybase.org http://ca.expasy.org/sprot/ http://www.cdfd.org.in/silksatdb http://www.cdfd.org.in/wildsilkbase/team.php http://www.ebi.ac.uk/ http://www.fruitfly.org https://www.genevestigator. ethz.ch. http://insects.eugenes.org/DroSpeGe/ http://www.issas.ac.cn http://www.jassilks.com http://kaiko2ddb.dna.affrc.go.jp http://morus.swu.edu.cn/morusdb http://www.naas.go.kr/ http://www.ncbi.nlm.nih.gov/ http://www.nias.affrc.go.jp http://pir.georgetown.eduwww.pubmedcentral.nih.gov/ http://resourcedb.nbrp.jp/resource/list.jsp http://sgp.dna.affrc.go.jp/index.html http://www.shigen.nig.ac.jp/silkwormbase/index.jsp http://silkbase.ab.a.u-tokyo.ac.jp/cgi-bin/index.cgi http://www.silkgermplasm.com |

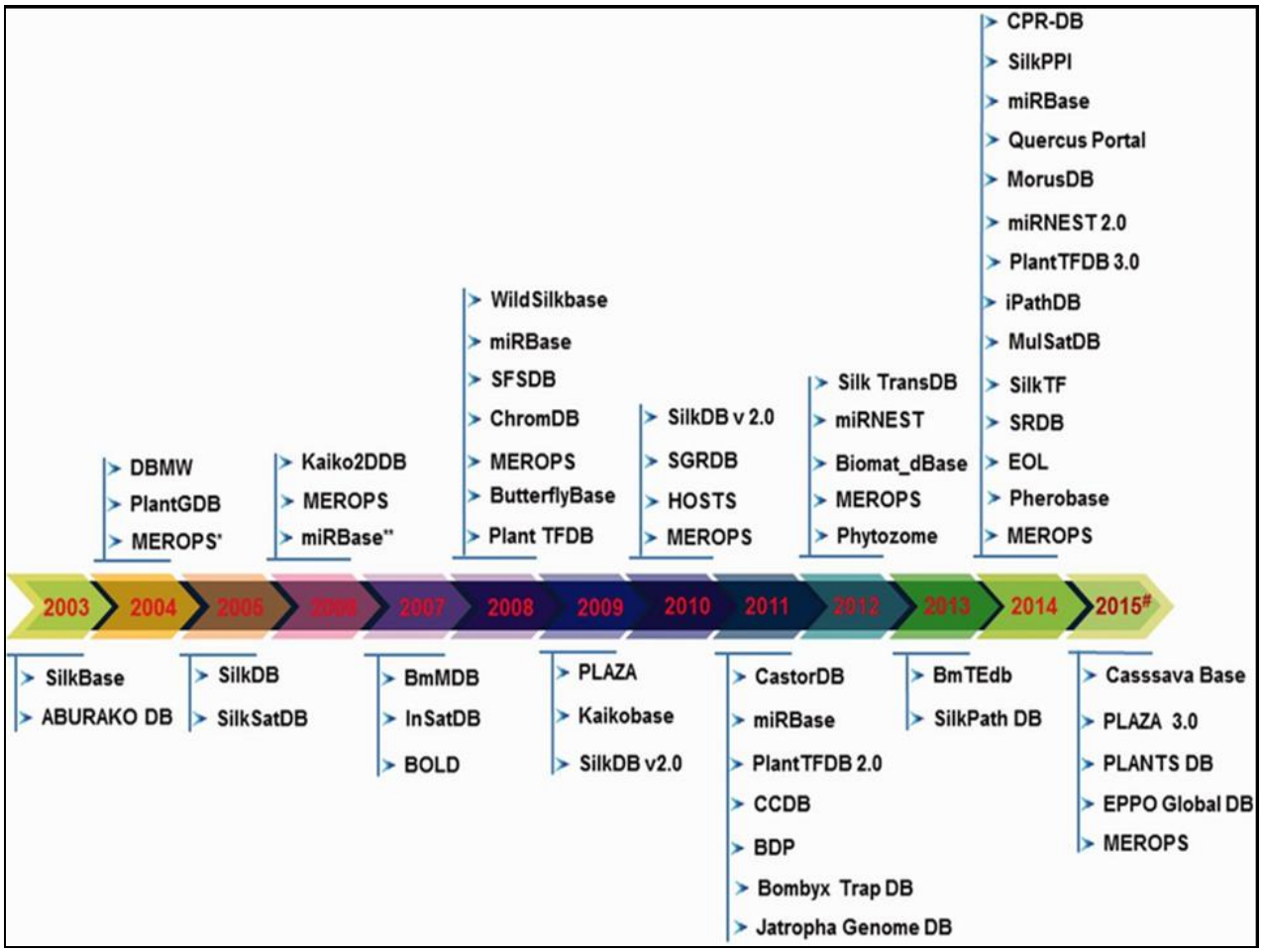


**Fig 1**

**Need for transgenesis in mulberry**

Mulberry is adversely affected by stresses like salinity and drought (Lal *et al*., 2008) [5]. The leaf water content may also drop in case of stress, which makes them inapt for silkworm rearing. At present, limited genomic resources are available which can be exploited for investigations pertaining to productivity enhancement in mulberry (Khurana and Checker, 2011) [3]. Previously, we reported transformation of mulberry with hva1 gene from barley for drought and salinity (Lal *et al*., 2008; Checker *et al*., 2011) [5, 3] and later, a tobacco osmotin

gene that generated abiotic as well as biotic stress tolerant mulberry (Das *et al*., 2010) [1]. Mulberry transgenics developed for tolerance against salinity and drought have the capability to reduce the dependence on water for growth and can also be raised in saline soils. Second generation mulberry transgenics were raised with the osmotin gene under a drought inducible promoter. These transgenics display abiotic stress tolerance and also tolerance against biotic fungal pathogens (Das *et al*., 2010) [1]. Overexpression of beta-carotene hydroxylase1 (BCH1) in *Morus indica* cv. shows higher levels of carotenoids and improved oxidative stress tolerance as compared with the untransformed wild type under non-stressed and stressed conditions. The BCH1 transgenics are found to be more tolerant to High light, UV irradiation and High temperatures by maintaining better membrane integrity under induced oxidative stress (Saeed *et al*., 2015) [6].

**First Transgenic mulberry by Paramjit Khurana**

Mulberry is adversely affected by stresses like salinity and drought (Lal *et al*. 2008) [5]. India has developed the first transgenic mulberry plant in the world with drought and salinity tolerance. The breakthrough was achieved in a laboratory in South Campus, Delhi University, where the transgenic plants are currently being tested for proper gene expression. The development of mulberry plant with transgenic gene HVA-1 is found drought and salinity resistant.

**Need for transgenesis in silkworm**

The loss of life of silkworms due to diseases prompted the researchers of CDFD and APSSRDI to develop transgenic silkworms resistant to BmNPV virus. These silkworms can be reared by the farming community through the year. A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. This is for the first time that the RCGM has given permission to conduct field trials on any animal or insect. Silkworms from China and Japan have the capacity to produce good quality silk but, if introduced in the tropical climate of India, they succumb to diseases as their resistance level is low. Of late, Indian agriculture has seen significant improvement in the production of bivoltine silk. However, the rearing of bivoltine silkworm hybrids is not possible through the year, leading to dependence on inferior cross-bred hybrids especially during adverse climatic conditions.

**Resistance of transgenic silkworm to BmNPV could be improved by silencing ie-1 and lef-1 genes**

Zhang *et al*. (2014) [13] reported that RNA interference (RNAi)-mediated viral inhibition has been used in several organisms for improving viral resistance. They reported the use of transgenic RNAi in preventing *Bombyx mori* nucleo polyhedrovirus (BmNPV) multiplication in the transgenic silkworm *B. mori*. They targeted the BmNPV immediate-early-1 (ie-1) and late expression factor-1 (lef-1) genes in the transiently transfected BmN cells, in the stable transformed BmN cell line and in the transgenic silkworms. They generated four piggyBac-based vectors containing short double-stranded ie-1 RNA (sdsie-1), short double-stranded lef-1 RNA (sdslef-1), long double-stranded ie-1 RNA (ldsie-1) and both sdsie-1 and sdslef-1 (sds-ie1-lef1) expression cassettes. Strong viral repression is observed in the transiently transfected cells and in the stable transformed BmN cells transfected with sds-ie-1, sdslef-1, ldsie-1 or sds-ie-lef. The decrease of ie-1 mRNA level in the sds-ie1-lef1 transiently transfected cells was most obvious among the cells transfected with different vectors. The inhibitory effect of viral multiplication is decreased in a viral dose-dependent manner, the infection ratio of transfected cells for sds-ie-1, sdslef-1, ldsie-1 and sds-ie-lef decreased by 18.83%, 13.73%, 6.93% and 30.63%, respectively, compared with control cells 5 days after infection. They generated transgenic silkworms using transgenic vector piggyantiIE-lef1-neo with sds-ie1-lef1 expression cassette, the fourth instar larvae of transgenic silkworms of generation G5 exhibited stronger resistance to BmNPV, the mortalities for the transgenic silkworms and control silkworms were 60% and 100%, respectively, at 11 days after inoculation with BmNPV (106 occlusion bodies per ml). This suggests that double-stranded RNA expression of essential genes of BmNPV is a feasible method for breeding silkworms with a high antiviral capacity.

**Precocious metamorphosis in transgenic silkworms overexpressing juvenile hormone esterase.**

Insect growth and development are intricately regulated by the titers of juvenile hormones (JHs) and ecdysteroids (or their metabolites) in the insect hemolymph. Hydrolysis of the methyl ester of JH by a JH-specific esterase (JHE) is a key pathway for the degradation of JH. Tan *et al*. (2004) [11] generated transgenic silkworm strains that overexpress JHE by using the binary GAL4/UAS system. Overexpression of JHE from the embryonic stage resulted in larval–pupal metamorphosis after the third stadium, two stadia earlier than that observed in wild-type insects. This precocious metamorphosis suggests that JHs are not critical for normal development of embryo or larva before the second molt in Lepidoptera (moths and butterflies).The transgenic approach is very important to study the function of key physiological events that occur from embryogenesis. Until now, these types of studies are possible only in later larval stadia by using physical techniques such as allatectomy or the application of JH analogues. With the introduction of transgenic silkworm hybrids, the quality parameters with reference to silk grade get improved as compared to the present commercial hybrids.

**Transgenic silkworms (*Bombyx mori*) produce recombinant spider dragline silk in cocoons**

The silkworm *Bombyx mori* also synthesizes large amounts of silk proteins in silk glands and spins them out as a fibrous thread to form a cocoon. Insights into the sequences and molecular structures have shown that silkworm silk is very similar to that of spider Dragline silk. For example, both of their genes have a high GC ratio, and the proteins are composed of tandem repeats that always contain Gly-rich and Ala-rich domains in their major parts. Wen *et al*. (2010) [12] reported that Spider dragline silk is a unique fibrous protein with a combination of tensile strength and elasticity, but the isolation of large amounts of silk from spiders is not feasible. The germline-transgenic silkworms (*Bombyx mori)* that spun cocoons containing recombinant spider silk. A piggy Bac-based transformation vector helps in carrying spider dragline

silk (MaSp1) cDNA driven by the sericin 1 promoter. Silkworm eggs were injected with the vector, producing transgenic silkworms displaying DsRed fluorescence in their eyes. Genotyping analysis confirmed the integration of the MaSp1 gene into the genome of the transgenic silkworms, and silk protein analysis revealed its expression and secretion in the cocoon. Compared with wild-type silk, the recombinant silk displayed a higher tensile strength and elasticity.

**Overexpression of host plant urease in transgenic silkworms**

Jiang *et al*. (2015) [2] Bombyx mori and mulberry constitute a model of insect-host plant interactions. Urease hydrolyzes urea to ammonia and is important for the nitrogen metabolism of silkworms because ammonia is assimilated into silk protein. Silkworms do not synthesize urease and acquire it from mulberry leaves. They synthesized the artificial DNA sequence urea's using the codon bias of *B. mori* to encode the signal peptide and mulberry urease protein. A transgenic vector that overexpresses ure-as under control of the silkworm midgut-specific P2 promoter was constructed. Transgenic silkworms were created via embryo microinjection.

**Generation of transgenic silkworms for production of erythropoietin in *Bombyx mori***

Prokaryote systems are not equipped with the protein maturation mechanisms necessary to generate eukaryotic proteins. In this sense, among the eukaryotes, silkworms have major merits in overcoming the difficulties. Such protein maturation mechanisms are available in silkworms. The transgenic silkworm producing rhEPO in the cocoon was generated and purified. Production of high quality silks is possible using transgenic silkworms. The transgenic silkworms produced by Japanese scientists. The transgenic silkworms produce silks having interesting green, red or orange fluorescence characteristics. To increase the production of fluorescent silk, these strains are back-crossed with the Japanese and Chinese parent races with selection in the successive generations. The generated silkworms produce the high quality of the recombinant silks having different characters. The most interesting silk possesses the fluorescence of the green, red and orange color. An efficient system for producing recombinant proteins is by using by transgenic silkworm. The system includes a method for creating the transgenic silkworm by injecting vector and helper plasmid DNA into preblastodermal silkworm eggs. The silkworm larva has the capacity to synthesize more than 0.5 g of silk protein during the final instar and expels the protein as a cocoon silk filament. The silk consists of two types of proteins, sericin and fibroin, which are synthesized in the middle silk gland (MSG) and posterior silk gland (PSG), respectively. Recently developed methods for producing recombinant proteins in the silkworm use the silk-synthesis systems in the silk gland and show that recombinant proteins can be produced very efficiently. The method developed in the PSG is suitable for the production of recombinant silk as a fiber for making fabrics and biomaterials for medical purposes.

**Molecular Cloning, Bioinformatic Analysis, and Expression of *Bombyx mori* Lebocin 5 Gene Related to Beauveria bassiana Infection**

Lu *et al*., 2016 cloned a full length c DNA of Lebocin 5 (BmLeb5) from silkworm *Bombyx mori* by rapid amplification of c DNA ends. The (BmLeb5 gene is 808bp in length. Bioinformatics analysis results showed that BmLeb5 owns an O-glycosylation site and four RXXR motifs as other Lebocins. Sequence similarity and phylogenic analysis results indicated that lebocins form a multiple gene family in silkworm as cecropins. Quantitative real-time PCR analysis revealed that BmLeb5 was highest expressed in the fat body in the silkworm larvae infected by *Beauveria bassiana*, the expression level of BmLeb5 was upregulated in the fat body and hemolymph which are the most important immune tissues in silkworm.

**Characterization of anti-CD20 monoclonal antibody produced by transgenic silkworms (*Bombyx mori*).**

In response to the successful use of monoclonal antibodies (mAbs) in the treatment of various diseases, systems for expressing recombinant mAbs using transgenic animals or plants have been widely developed. The silkworm (*Bombyx mori*) is a highly domesticated insect that has recently been used for the production of recombinant proteins. Because of their cost-effective breeding and relatively easy production scale-up, transgenic silkworms show great promise as a novel production system for mAbs. Tada *et al*. (2015) [10] established a transgenic silkworm stably expressing a human-mouse chimeric anti-CD20 mAb having the same amino acid sequence as rituximab, and compared its characteristics with rituximab produced by Chinese hamster ovary (CHO) cells (MabThera®). The anti-CD20 mAb produced in the transgenic silkworm showed a similar antigen-binding property, but stronger antibody-dependent cell-mediated cytotoxicity (ADCC) and weaker complement-dependent cytotoxicity (CDC) compared to MabThera. Post-translational modification analysis performed by peptide mapping using liquid chromatography/mass spectrometry. There was a significant difference in the N-glycosylation profile between the CHO- and the silkworm-derived mAbs, but not in other post-translational modifications including oxidation and deamidation. The mass spectra of the N-glycosylated peptide revealed that the observed biological properties were attributable to the characteristic N-glycan structures of the anti-CD20 mAbs produced in the transgenic silkworms that means the lack of the core-fucose and galactose at the non-reducing terminal. Hence transgenic silkworm have a promising expression system for the tumor-targeting mAbs with higher ADCC activity.

**Discovery of G protein–coupled receptors**

Sumathy *et al*. (2012) [9] reported that G protein–coupled receptors (GPCRs) are largest integral membrane proteins that communicate signals across the cell membrane through their interaction with heterotrimeric G proteins and regulate many of physiological processes of *Bombyx mori* such as neurotransmission, growth, development etc. The GPCRs of

the silkworm are identified using the computational methods and are classified as Rhodopsin-like receptors (Class A), secretin receptors (Class B), metabotropic glutamate/pheromone receptors (Class C), fungal mating pheromone receptors (Class D), cyclic AMP receptors (Class E), and frizzled/smoothened GPCRs (Class F). This provides a structural understanding at the atomic level of three-dimensional structure of silkworm serotonin receptor protein and their binding-sites and to elucidate of many promising active lead compounds.

**Production of the BmCecB1 antimicrobial peptide in transgenic silkworm**

*Bombyx mori* cecropinB1, this peptide has antibacterial activity against several Gram-positive and Gram-negative bacteria. *Bombyx mori* cecropinB1 (BmCecB1) is antimicrobial peptides from *Bombyx mori* and belongs to cecropin family. Antimicrobial peptides are important components of the innate immune systems in all living organism. To produce the BmCecB1 antimicrobial peptide, Kim *et al*. (2015) [4] constructed transgenic silkworm that expressed BmCecB1 4gene under the control BmA3 promoter using piggyBac vector. The use of the 3xP3-driven EGFP cDNA as a marker allowed them to rapidly distinguish transgenic silkworm. Mixtures of the donor vector and helper vector were micro-injected into 600 eggs of bivoltine silkworms, Baegokjam. In total, 49 larvae (G0) were hatched and allowed to develop into moths. The resulting G1 generation consisted of 22 broods, and they selected 2 broods containing at least 1 EGFP-positive embryo. The rate of successful transgenesis for the G1 broods was 9%.They identified 9 EGFP-positive G1 moths and these were backcrossed with wild-type moths. With the aim of identifying a BmCecB1 as antimicrobial peptide, they investigated the Radical diffusion Assay (RDA) and then demonstrated that BmCecB1 possesses high antibacterial activities against Gram negative bacteria. Hence BmCecB1 peptide may be useful as a

**Silkworm genome project**

The domesticated silkworm, *Bombyx mori*, has long been used as a model system for basic studies because of its large body size, ease of rearing in the laboratory, and economic importance in sericulture. The well-developed genetic resources of this species include more than 400 described mutants, which have been mapped to >200 loci, comprising 28 linkage groups. The full genome of the silkworm was published in 2008. Draft sequences were published in 2004. The sequences are assembled by RAMEN, a newly developed software program for large-scale whole-genome shotgun sequencing. RAMEN basically follows the overlap layout consensus paradigm, but individual steps have been accelerated by novel or state-of-the-art software implementation ideas such as lookup table generation of seed strings for highly sensitive and rapid detection of overlapping reads, precise alignment by efficient banded dynamic programming, a repeat untangling method of transforming a repeat subcontig flanked by two unique subcontigs into one unique contig, and an efficient multiple alignment algorithm utilizing seeds in the lookup table. By using the newly developed RAMEN assembler, the sequence data derived from whole-genome shotgun (WGS) sequencing are assembled into 49,345 scaffolds that span a total length of 514 Mb including gaps and 387 Mb without gaps. Because the genome size of the silkworm is estimated to be 530 Mb, almost 97% of the genome has been organized in scaffolds, of which 75% has been sequenced. By carrying out a BLAST search for 50 characteristic *Bombyx* genes and 11,202 non-redundant expressed sequence tags (ESTs) in a *Bombyx* EST database against the WGS sequence data.

**Conclusions**

With the advent of genomic and proteomic research from bacteria to man an unprecedented data generated are pertinently analyzed and managed by the evolving science-bioinformatics. In scientific research, *Bombyx mori* L. is considered as a model insect for molecular studies along with the fruit fly (*Drosophila melanogaster*) and a central model species for genome studies in moths and butterflies (the insect order Lepidoptera). As a consequence, new findings in the fields of proteome, genome and bioinformatics have resulted in the exponential generation of data that are stored in assorted array of databases. These databases not only reducing the gap and time while allowing information's to be accessed also emerged as a highly valuable platform through which scientific community can use, exchange and analyze molecular data across the world on mouse click.

The computational approaches in various biological disciplines including agriculture/sericulture is not merely a reflection of a general extended usage of computers and the internet, but due to the creation of useful databases coupled with appropriate software's and methods for access by the rest of the scientific community with ease. Application of bioinformatics tools and techniques not only facilitated detection of proteomic and genomic diversity among the species/strains but that resulted in finding a gap in the silkworm genome sequence of a strain that diverged during the course of domestication. Computational Biology has emerged as one of the leading interdisciplinary realms having applications and analytical implications in every branch of biological sciences. The efforts to understand silkworm or its interactions with other organisms have generated a plethora of information which has been converted into different types of electronic databases. Application of modern methodologies, such as, next generation sequencing - comparative modelling and simulation, docking and design of specific molecules which not only provide insight on disease incidence and progression in silkworms but also provides us with necessary information for enhancement of silk fibre quality for enhancing that silken touch. These Seri-bioinformatics databases are a valuable seri-bioresource.

**Conflict of interest**

There is no conflict of interest among the authors.

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