Nucleus (2022) 65:129–150
https://doi.org/10.1007/s13237-021-00355-2

REVIEW ARTICLE

Application of biotechnology in sericulture: Progress, scope and prospect

Khasru Alam[1] · V. S. Raviraj[2] · Tanmay Chowdhury[1] · Anil Bhuimali[1] · Parthadeb Ghosh[3] · Soumen Saha[1]

Received: 27 October 2020 / Accepted: 24 May 2021 / Published online: 7 June 2021

Abstract

Traditional sericulture represents rearing of silkworm for production of silk that provides livelihood opportunity to millions of people in the country besides earning foreign exchange. The time has come to diversify the whole sericulture process for meaningful realization of its output under the present day scenario. The advent of modern biotechnology and its application have opened a new arena of the synthesized science for silk production. The vast potential of silk industry can effectively be exploited by the application of modern day biotechnological approaches like, marker assisted selection and expression of foreign protein through transgenic approaches. On the other hand, the silk quality has been enhanced using probiotics and providing artificial feed to the silkworm. The potential of silk has been further exploited for biomedical applications. In this communication the comprehensive account of biotechnological applications in sericulture and its byproducts for the development of sericulture industry are compiled while emphasizing the need of applying modern biotechnology for meaningful growth and development of sericulture and silk industry.

Keywords Sericulture · *Morus alba* · *Bombyx mori* · Molecular markers · Transgenic · Silk

Introduction

Sericulture, the synthesized science of silk production provides employment opportunities to around 6 million people of the country in its entire value chain extending from soil to silk. Since independence, India has increased its silk production from 11,000 MT in 1989–1990 to 31,906 MT in 2017–2018 [203] and has become the second largest producer in the globe. The major thrust areas for sericulture are the production of superior mulberry and silkworm varieties for the tropical conditions of the country, identification of new silkworm rearing technologies, suitable methods for containment of pests and diseases in sericulture etc. [195]. Owing to changing climate and complex farming conditions of the sericluture farmers, focus needs to be shifted from the traditional interventions to application of advanced scientific knowledge. Applicability and potentiality of biotechnology in increasing the productivity of sericulture have been acknowledged unequivocally in the recent past in many scientific discussions. It has been proved to be a powerful tool for sustainable growth and development of sericulture and silk industry. Silk is one of the most precious natural fibers and the unit price of raw silk is roughly estimated to be twenty percent more than raw cotton [132]. Increased demand of natural fibre, application of silk as biomaterial as well as silkworm as a model organism for the production of foreign protein encouraged the scientific community to explore biotechnology among many other techniques to improve production and application of silk for both textile and non textile industrial purposes.

In the recent past, research contribution of various organizations enabled Indian sericulture industry to make a good

Corresponding Editor: Bani Gajra; Reviewers: Gaurab Gangopadhyay, Devendra Kumar Pandey.

✉ Soumen Saha
thustu@gmail.com

[1] Cytogenetics and Plant Biotechnology Research Unit, Department of Sericulture (Centre for Applied Biology), Raiganj University, Uttar Dinajpur, Raiganj 733134, West Bengal, India

[2] Silkworm Breeding and Genetics Lab, Central Sericultural Research and Training Institute, Central Silk Board, Ministry of Textiles, Government of India, Berhampore, West Bengal 742101, India

[3] Cytogenetics and Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Nadia, Kalyani 741235, West Bengal, India

stride by way of introducing new and improved mulberry and silkworm varieties, improved silkworm rearing technologies for tropical conditions and management strategies of diseases and pests in sericulture [226]. Even though conventional breeding approaches in sericulture have successfully introduced many productive breeds and hybrids, but their full potential could not be utilized in the field due to adverse agro climatic conditions and infections by pathogens while practicing repeated rearing. Situation worsens further because of limited ground water, soil differentiation with poor native fertility and improper cultivation practices [213]. The use of modern biotechnological tool for improvement of silkworm, host plant, development of artificial feed, seri waste utilization, production of silk-protein based biomaterials etc. holds great potential not only to meet production demand of silk but also to improve the industry in a comprehensive manner. On the other hand, *Morus alba* which is rich in folic acid, carotene, vitamins, flavonoids, tannins, saponins, ascorbic acid and antioxidents with phytoconstituents such as anthocyanins, anthroquinones, glycosides and oleanolic acid has been explored using biotechnological applications for its pharmacological significance. Recent advances have reported use of *Morus alba* as antidiabetic, anticancerous, immunomodulatory and antimutagenic agents. The possible areas of biotechnological interventions in sericulture are presented in Fig. 1. The plethora of information provided by the several genome projects, advances in functional genomics and transgenic technologies have added new insight into the field of seri-biotechnology. The potential application of these new approaches into sericulture may bring us closer to achieving the growing demands of silk in the country. Therefore, present review is an attempt to focus on the applicability of different modern biotechnological approaches like molecular marker system, transgenic technologies, development of artificial feed, immunodiagnosis techniques, expression of foreign protein in silkworm,

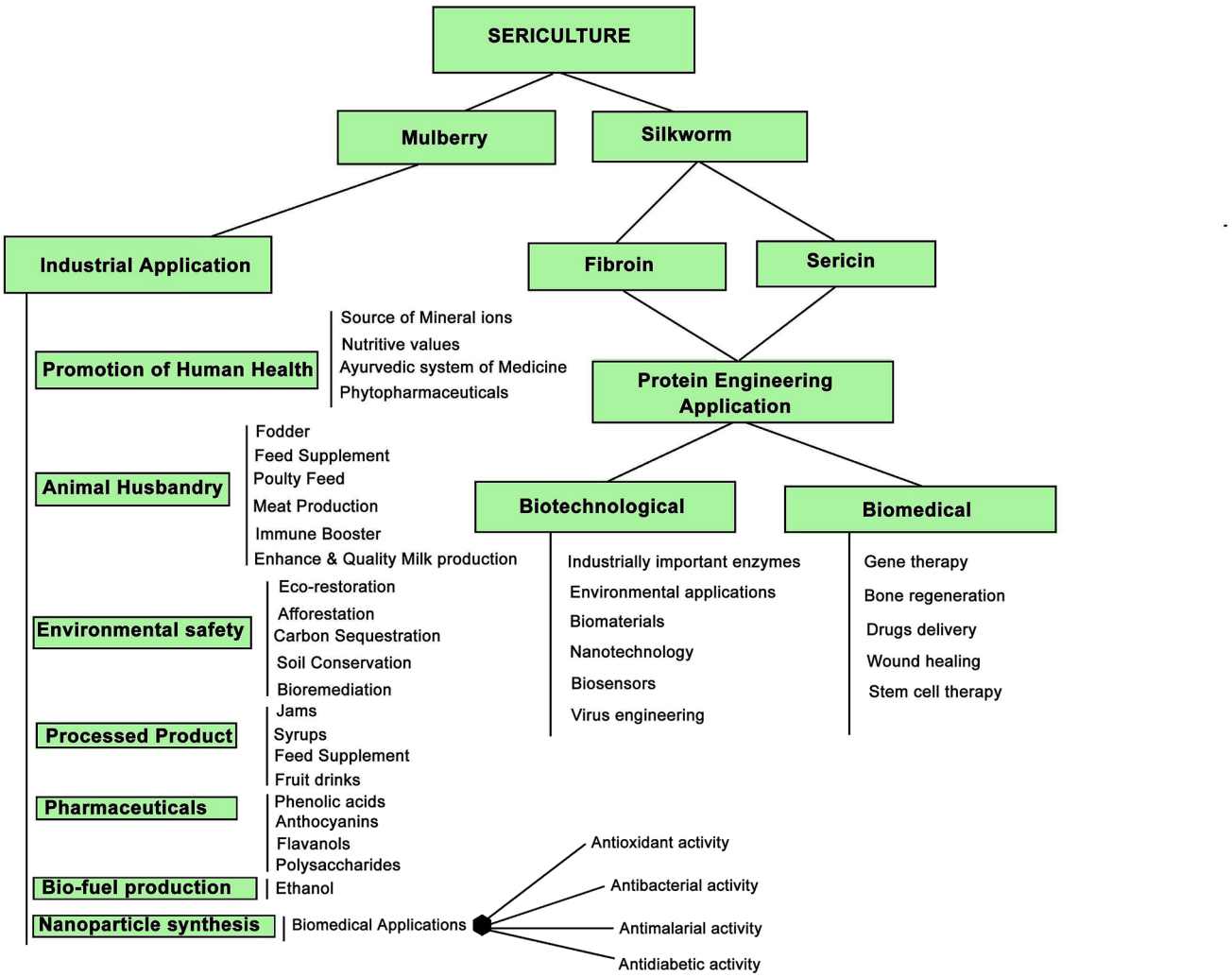

Fig. 1 Schematic representation on the possibilities of biotechnological application in sericulture

development of silk-based biomaterials and control of diseases, pests etc. in sericulture.

Molecular markers for mulberry improvement

The principal application of mulberry plants in sericulture is its leaves which are used for silkworm rearing to produce silk. According to an estimate on the economic return of sericulture point of view, mulberry cultivation cost alone constitutes more than 60% of the total expenditure for the entire cocoon production [56]. This further necessitates development of mulberry varieties with high leaf yielding potentials. Traditional mulberry breeding strategies have evolved several mulberry high yielding varieties viz. S-1, K-2, Kokuso-13, Shin-ichinose, V-1, S-13, S-34, S-799, S-30, S-36, S-54, Tr-4, G-9, RC-1, RC-2 [192, 210, 223]. But mulberry breeders encountered several problems in the breeding programmes, like limited information on genetics and inheritance pattern of mulberry, absence of pure lines, lack of definite parents, dioecious nature, inbreeding depression, perennial nature with long juvenile period, lack of genetic markers and efficient screening strategies [236]. Most of the yield and associated traits like leaf qyality and yield, resistance to abiotic (water, alkalinity, frost, etc.) and biotic stress (root rot, root knot nematode, whitefly, tukra etc.), efficiency in nutrient uptake capacity. Leaf quality of mulberry is polygenic in nature and influenced highly by genotype and environment interaction while manifesting its characteristics [239]. Therefore, situation demands application of modern biotechnological tools like use of molecular markers, transgenesis for introduction and over expression of desirable genes or silencing of undesirable genes through RNA interference technology [250].

Molecular marker (DNA/RNA) is a specific physical unit on a chromosome whose inheritance pattern can be observed and studied well. Exploration of mulberry genome has created *Morus* database which is a boon in identifying the vital regions of mulberry genome for exploiting in breeding process. Morus DB—Mulberry Genome Database (https://morus.swu.edu.cn/) is one of the sites where all the details of genetic makeup and molecular markers such as SNPs, ISSRs, RFLPs can be accessed.

Molecular markers enable fast and accurate identification of breeding lines, hybrids, cultivars and species, facilitate analysis of genetic diversity and also allow establishment of phylogenetic relationship with more precision than that was previously possible with morphological and biochemical techniques. The DNA markers are extensively used for screening genotypes, identification of gene of interest, characterization of germplasm collections and other gene related studies in mulberry [73, 122]. There are different DNA markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) [125]. Marker assisted selection (MAS) has got many advantages over conventional breeding methods as it enables the breeder to select desirable hybrids at the seedling stages, free from environmental interference, time saving, accurate and precise. MAS provide the potential for improving selection efficiency by allowing early selection, reduced number of generations and population size [208]. Genetic diversity, molecular characterization of germplasm and varieties, development of linkage and quantitative trait locus (QTL) map, association mapping, parental selection schemes and marker-assisted selection (MAS) are some of the important areas where molecular markers can play a great role for improvement of mulberry plants [105, 251].

There are plenty of reports on application of molecular markers for improvement of mulberry. Application of molecular markers namely RAPD [10, 42, 93, 141, 155–157, 165, 167, 225, 262, 274] AFLP [85, 92, 198] ISSR [98, 117, 240, 243, 245, 247, 256, 261, 275, 276, 277] RAPD with ISSR [19, 27, 46, 47, 51, 86, 94, 189, 200, 206, 238, 242, 244, 248, 263] RAPD, ISSR and SSR [12, 78] has been reported by many authors. Markers like genomic-SSR [109, 159, 166, 170, 257, 258], sequence-related amplified polymorphism (SRAP) [278], internal transcribed spacer (ITS) [260, 266], start codon targeted polymorphism (SCoT) [185], Cleaved Amplified Polymorphic Sequences (CAPS) [17], direct amplification of minisatellite DNA (DAMD) [33] were also used not only to ascertain molecular diversity in cultivated and wild mulberry species but also to identify its route of introduction and proliferation in India. Studies on molecular profile for genotypes and estimation of the genetic diversity among different germplasm collections of mulberries also resulted identification of SSR markers and SNPs from different mulberry species [148, 188]. The details of molecular markers used in mulberry improvement during last two decades have been listed in supplementary table (Table A).

Introduction of marker assisted breeding in mulberry further got a new momentum when whole-genome shotgun sequencing of haploid mulberry species (*M. notabilis*) was reported, the draft genome of 357 Mb included 128 Mb repetitive sequences with 27,085 high confidence protein coding loci in tandem with complete gene structure [82]. Attempts were made to develop QTL (Quantitative Trait Locus) maps that are specific to important agronomic traits such as water use efficiency (WUE), root traits and yield attributing characters in mulberry, using dominant markers such as RAPD and ISSR. These resulted few markers linkages in the QTL map governing the character of interest [139, 158]. But identification of marker intricately linked to the particular trait is still lacking in mulberry. Table 1

Table 1 Molecular maps developed in mulberry

Molecular map	Pedigree of mapping population	Markers	Heredity nature of marker	Agronomic trait targeted	References
Genetic linkage map	S-36 × V-1	RAPD, ISSR, and SSR	Dominant, co-dominant	No	[237]
QTL map	V-1 × Mysore Local	RAPD and ISSR	Dominant	Yield contributing traits	[158]
QTL map	Himachal Local × MS-3	RAPD and ISSR	Dominant	Water use efficiency	[139]
QTL map	Dudia White × UP	RAPD and ISSR	Dominant	Root traits	[139]
Genetic linkage map	Dudia White × UP-105	SSR	Co-dominant	No	[224]

depicts some of the significant attempts on the development of molecular maps in mulberry improvement.

Improved mulberry characters using molecular markers in India

Molecular markers assisted breeding has benefited the Indian sericulture in many ways. The researchers in India have indentified many RAPD and ISSR markers associated with genetic diversity leading to development of desirable characters targeting specific gene/proteins encoding such as high leaf yields, abiotic stress resistance and, drought resistance using over expression of certain genes through molecular approach. The *Morus notabilis* genome database (https:/morus.swu.edu.cn) published in 2017 will help to extrapolate new primer design and their application in generatinge linked markers with quality traits.

Conventional breeding and molecualar breeding

Breeding is an important method to maintain silkworm races based on their characters and phenotypes. It also provides us the base material for development of new breeds specific to a particular region or climate, Conventional breeding does not establish a particular character improvement in organism. On the other hand, molecular breeding helps us establish specific characters in a species using molecular markers associated with desired phenotype of the organism in which one is interested. Outcome of this molecular breeding in turn will help in increasing the productivity of that specific phenotypic character item in the sericulture industry.

Tissue culture in mulberry genetic improvement

The main applications of plant biotechnology are to promote conservation, diversification, and sustainable use of plant genetic resources for food and agriculture besides improving food security and fostering socio-economic development. The role of plant biotechnology and its use of advanced methods in sericulture is to improve the productivity trait and their survivability under stress conditions. However, perennial nature of the plant coupled with prolonged juvenile period slow down the process of mulberry improvement [103]. Further, cross-pollination is the rule rather than an exception, enormous heterozygosity occurs in the plant [57]. The diploid (2n) chromosome number of the species is 28, but highly polyploid numbers, up to 308, can be found. Among the polyploids, the triploids have many desirable traits, including quality, and resistance to cold and disease. However, the production and multiplication of triploids are time consuming [57].

Mulberry can be vegetatively propagated through stem cuttings, grafting or budding. However, success of these methods depends on several factors such as genetic makeup of the plant, age and physiological conditions of the parental cutting, climatic conditions and others. Additionally, newly developed mulberry varieties cannot immediately be propagated through stem cuttings as at least 6–7 month maturity is required to make the cuttings from the parental plant [96]. Moreover, the dioecious nature of mulberry and the occurrence of genetic linkage with both desirable and weak traits limit improvements that can be attained through conventional hybridization.

The emerging application of tissue culture technology to clonal propagation provides an alternative to the routine vegetative propagation of woody species with such desirable traits [36, 222]. In fact, the in vitro culture of cells, tissues, and organs offers unparalleled opportunities for tree improvement [101]. Regeneration of plants via tissue culture is based on the principle of totipotency originally proposed by Haberlandt in 1902 [234]. Plant cell and tissue cultures play an important role in the manipulation of plants for improved crop varieties. Plant regeneration systems are an essential part of micropropagation and molecular approaches leading to plant improvement in mulberry. The possibility for qualitative and quantitative improvements in mulberry varieties is vital to the advancement of the sericulture industry.

As we know tissue-culture techniques have been used extensively for mass propagation of forest and cultivated trees, including plantation crops [24]. Plant tissue culture has found its applications not only in basic and applied research but also in commerce and various trades. In vitro regeneration of whole mulberry can be achieved using diverse types

of explants such as meristems/buds, protoplast-dervived somatic hybrid, embryo, ovule, anther, and cells. Over the past two decades many reports have surfaced concerning plant regeneration from different explants of mulberry which have been detailed in Table 2. Tissue culture techniques such as, micropropagation, callus culture, organogenesis, somatic embryogenesis, and somatic hybridization were employed to improve the qualities of mulberry plant. Additionally, in vitro screening of genotypes for stress tolerance and yield, production of haploids and triploids, and improvement of secondary metabolites through transgenic approach are recomended.

Molecular markers in silkworm improvement

The art of silkworm rearing for silk production in our country is an ancient culture and even today sericulture constitutes an essential component of the rural economy in India [60]. The country is bestowed with extraordinarily rich silkworm genetic base in the form of mulberry silkworms and wild silk producing insects. Despite having such a wide silkworm genetic base, information is hardly available either on the unique features of many of these genotypes, or to the extent of genetic diversity between or within the genotypes/races. Till date, genetic improvement of these precious insects is usually ascertained based on their morphological characteristics which, are highly biased and environmentally dependent, thus requiring an authentic technique like MAS for genotype characterization and evaluation. As far as mulberry silkworm, *Bombyx mori* L., is concerned, more than 400 visible mutations have been placed in the linkage maps [64] which represent 217 loci consisting of mostly morphological and a few isozyme markers. Research on genetics of silkworm across the world has helped in establishing silkworm genome database called silkbase (http://silkbase.ab.a.u-tokyo.ac.jp/cgi-bin/index.cgi). This has become an integral part in exploring the genome of silkworm for improvement of sericulture industry. Exploring genome of silkworm has revolutionized the sericulture industry by identifying the molecular markers using linkage mapping. This led in identifying key regions in the genome of silkworm for specific phenotypes that have changed the face of the silkworm breeding. Hence, a lot of work on DNA based genetic markers in silkworm started emanating in the 1990s and a preliminary linkage map of 169 loci using RFLPs [201] and RAPD [172] was constructed. Studies on PCR-based markers, RAPD markers, and DNA fingerprinting with minisatellite probes in respect to Indian silkworm have also been carried out [152]. Further, thirteen silkworm strains were used for genetic characterization using inter simple sequence repeats (ISSR). The ISSR-PCR produced 39 fragments of which 76.98 per cent were polymorphic and the diversity index was observed to be 0.957 percent [153]. Afterwards, for complete genome analysis of the silkworm, *Bombyx mori* L., expressed sequence tag (EST) database was constructed [142, 143] covering about 55% of all the genes of silkworm. To identify and mapping of sex-linked traits in the silkworm genetic mapping of Z chromosome and identification of W chromosome- specific markers in the silkworm were carried out [151]. Fang et al. [72] while studying genomic analysis of cocoon and associated traits in silkworm through restriction-site-associated DNA sequencing (RAD-Seq) reported identification of a total of 11 cocoon yield-related QTLs on 7 chromosomes using the composite interval mapping (CIM) algorithm. The tasar silkworm studies also reveal that molecular markers are always better in identifying the genetic makeup, determining population structure and interrelationship among different ecoraces of *Antheraea mylitta*. Rao et al. [181] while studying molecular characterisation of Daba and Andhra ecoraces of tasar reported occurrence of certain common bands in both the ecoraces indicating possible genetic relationship between these two ecoraces. Chatterjee et al. [41] reported considerable genetic variation through ISSR markers in six different wild tasar ecoraces. Considerable intra and inter population diversities through ISSR markers within and among semi domesticated Daba bivoltine, Daba-trivoltine and nature grown Daba were also reported by Kar et al. [97]. Their report further suggested that semi domesticated population of Daba ecorace was at the threshold level of discriminating themselves. Genetic diversity between and within population of Raily, Daba and Modal was also studied by using 12 ISSR and 10 RAPD primers [246]. Phylogenetic relationships of nine tasar silkworm ecoraces were reported by Mahendran et al. [129] by RFLP method. Population structures of eight different ecoraces using ten amysat microsatellite loci were studied by Chakraborty et al. [38].

Exploration of the silkworm genome has helped to identify specific markers for thermotolerance, no glue eggs, silkworm resistance to viruses, regulation of moulting, regulation of body shape etc. Silkworm research in India by Central Silk Board has made advances by successfully identifying microsatellite markers linked to thermotolerance by Bulk Segregation Analysis [39]. Thermotolerant silkworm is under development in India for the tropical environment. Based on the available data we have summarized the important development of molecular map and use of markers in improvement of silk work in Tables 3 and 4.

Transgenics research in sericulture

Advancement of modern biotechnology enables us to identify a gene of interest that can be separated, cut off, inserted, transformed, and expressed into a foreign system. Such genetic manipulation is referred as genetic engineering. Knowledge on modern biotechnology has made it possible

Table 2 Experiments on in vitro propagation in mulberry (*Morus* spp.) and the results during last 20 years

Species	Genotype/cultivar	Explants	Country	Remarks	Year/[References]
M. alba L	S-1	Leaf	India	Plant transferred to soil	2000 [241]
M. alba L	S-36	Endosperm	India	Plant transferred to soil	2000 [221]
M. alba L	Chinese White, Kokuso, Ichinose	Leaves, petioles, intermodal segments	India	Plant transferred to field	2001[34]
M. indica L	K-2, DD	Hypocotyl, cotyledon, leaves, petioles, internodal segments, roots	India	Plant transferred to field	2001[31]
M. alba L	Chaina White	Nodal segments	India	Plant transferred to soil	2002 [48]
M. indica L	M-5, S-36, S-13				
M. latifolia Poilet	–	Nodal segments	Taiwan	Plant transferred to field	2002 [123]
M. alba L	M-5	Mature zygotic embryo,	India	Primary zygotic embryos	2002 [2]
M. alba L	–	Shoot tips, Nodal segments	India	Plant transferred to soil	2003 [11]
M. alba L	Chaina White, Kokuso-27, Ichinose	Shoot tips, Nodal segments	India	Plant transferred to soil	2003 [35]
M. multicaulis Perr	Goshoerami, Rokokuyaso				
M. alba L	S-1, K-2	Nodal segments	India	Plant transferred to soil	2003 [220]
M. alba L	–	Primary somatic embryos	India	Secondary embryoids	2004 [4]
M. indica L	M-5, S-36, S-13	Nodal explants	India	Plant transferred to soil	2005 [49]
M. alba L	China White				
M. indica L	S-36	Leaf-derived protoplast	India	Plantlets transferred to green house	2005 [231]
M. alba L	Sujanpur	Nodal segments	India	Plants transferred to soil	2006 [102]
M. alba L	Local, Sujanpur	Nodal segments	India	Plants transferred to soil	2007 [7]
Morus spp.	–	Side bud	China	Organogenesis	2008 [88]
Morus sp.	DD, K-2, AR-12, MR-2	Leaf, epicotyls, hypocotyl	India	Organogenesis	2009 [177]
M. alba L	Mysore-5	Nodal segment, Axillary bud	India	Oraganogenesis	2009 [26]
M. indica L	S-36, V-1	Nodal segment	India	Callus formation, Plant transferred to field	2010 [182]
M. nigra L	–	Nodal segment	India	Plant transferred to field	2011 [272]
M. alba L	S-1	Nodal segment	India	Plantlets transferred to soil	2011 [43]
M. indica L	V-1	Nodal segment	India	Plantlets transferred to green house	2011 [191]
M. macroura Miq	–	Nodal segment	Pakistan	Callus formation, Plant transferred to field	2012 [9]
M. indica L	S-1635	Nodal segment	India	Callus, Flower bud	2013 [112]
M. indica L	V-1	Leaf	India	Plantlets transferred to soil	2013 [178]
M. alba L	S-1	Nodal segment	India	Plant transferred to field	2016 [189]
M. indica L	K-2	Nodal explants	India	Plantlets transferred to green house	2017 [77]
M. nigra L	Black mulberry	Axillary buds	India	Plant transferred to field	2018 [63]
M. multicaulis Perr	Goshoerami	Nodal explants	India	Plant transferred to field	2018 [184]
M. cathayana Hemsl	–	Nodal segments	Indonesia	Shoot multiplication	2019 [264]
M. nigra L	Portuguesa	Apical and axillary buds	Brazil	Plantlets transferred to green house	2019 [67]
M. alba L	White mulberry	Cuttings	Iraq	Organogenesis	2019 [80]
M. alba L	Yue 11, Sha 2×lun 109, Morittina, Kokuso 27, and Kanva- 2	Apical shoots, Lateral buds	Egypt	Plant transferred to soil	2020 [215]

Table 3 Molecular maps developed in Silkworm *Bombyx mori* L

Molecular map	Strains	Markers	Heredity nature of marker	References
Genetic linkage map	p50 × C108	RAPD	Dominant	[172]
Genetic linkage map	p50 × C108	RFLP	Co-dominant	[210]
Genetic linkage map	169 (*p*:39 + *p*:130) × 186 (*p*:44 + *p*:142)	RAPD and EST	Dominant and Co-dominant	[270]
Genetic linkage map	p50 × C108	STSs and RAPD	Co-dominant and dominant	[271]
Genetic linkage map	782 × od100	AFLP	Dominant	[218]
Scanning linkage analysis (SLA)	RF02 × RF50	RFLP	Co-dominant	[91]
Molecular linkage map	RF02 × RF50	RFLP	Co-dominant	[162]
Molecular linkage map	Nistari × NB_4D_2	SSR	Co-dominant	[171]
Genetic linkage map	Dazao, C108, JS, L10, F50B, 54A	SSR	Co-dominant	[134]
Genetic linkage map	p50T × C108T	SNP	Co-dominant	[268]
BAC-integrated linkage map	p50T × C108T	EST and RFLP	Co-dominant	[267]
Genetic linkage map	P107 × Khorasan Lemon	AFLP	Dominant	[137]
QTL linkage map	Nistari, Fa50B, Jingsong, Lan 10	SSR	Co-dominant	[273]
QTL based Genetic linkage map	P107 × Khorasan Lemon	AFLP	Dominant	[138]
Linkage and mapping analyses	P50 × H9	SSR	Co-dominant	[280]
Genetic linkage map	P50 × H9	SSR	Co-dominant	[259]

to improve our understanding on the living organisms and to apply the knowledge to the life and activities of mankind which include food, agricultural production, forestry, fish production, animal rearing and horticulture etc. [70, 164]. Modern biotechnology, especially genetic engineering enables us to obtain organisms carrying a gene or genes of interest from an unrelated organism. Scientists are taking advantage of recombinant DNA technology for the development of transgenics due to the limitations of conventional breeding in introducing desired characters in both mulberry and silkworm. Transgenic research has introduced desirable characters in silk and its associated products. Spider silk is one such example that has changed the silk industries intensely.

Transgenic mulberry

Mulberry is a woody tree species. Several protocols have been standardized so far for a time phased screening strategy to transform *Morus indica* L. using particle bombardment [32] and via *Agrobacterium tumefaciens* [30], yet there is a lot of difficulties in handling the same. Mulberry is very much susceptible to stresses like salinity and drought and leaf moisture content drops drastically while countering these stresses [111]. It has been observed that mulberry transgenics developed with the *osmotin* gene under a drought inducible promoter which showed abiotic stress tolerance and tolerance against biotic fungal pathogens [58]. Development of transgenic mulberry with *Hva1* gene from barley for drought, salinity and cold tolerance has been reported [44, 111]. Some of the significant attempts of mulberry improvement through genetic engineering have been enlisted in Table 5.

Transgenic silkworm

In silkworm also, germline transformation of *Bombyx mori* L. using microinjection of *PiggyBac* (PB) derived vectors and its optimization was elaborated extensively Tamura and his associates [217] have developed a transgenic silkworm using the DNA transposon *PiggyBac* as a vector for inserting the target gene into the silkworm chromosome. Use of transgenic silkworms to produce different recombinant protein as well as for produce high quality silks employing silk-gland as bioreactor have also become a reality now a days [108, 227, 279]. The ongoing development of transgenic silkworm for monoclonal antibody drugs for cancer [119, 214], orphan disease drugs and animal drugs has also been happening in recent times. Therefore, attempts towards developing genetically modified silkworms may lead to many innovations in sericulture and sericology [150]. Production of recombinant human serum albumin (HSA) in transgenic cocoon, production of glycoproteins with reduced antigenicity in transgenic silkworms are some of the recent advances paving new avenues of transgenic research in sericulture [135, 173].

Limitation of transgenesis

Although there have been many advantages of transgenesis in improving silkworm characters and mulberry plants characters, it comes with certain risks. The disadvantages are the possible risks of such transgenes escaping in the nature and blend through gene flow with naturally occurring population, which can lead to undesirable character in the organisms.

Table 4 List of Molecular markers used in Silkworm *Bombyx mori* L. during last 20 years

SL. No	Marker type	Silkworm races/hybrid/stain	Country/region	Remarks	Year/[References]
1	RAPD, SSR and RFLP	Hu204, KA, NB_1, NB_7, NB_{18}, NB_4D_2, Gungnong, Sarupat, Moria, C. nichi, Pure Mysore, Nistari, Daizo	India	Genetic analysis	2001 [153]
2	RAPD	Nistari, C'Nichi, Unknown-Karnataka, NB1, N124.C124, C108, Boropolu, Hu204, Chinese hybrid, Chinese Golden-70, Nan Naung 6A, Tashkahashi-112, Jam-23, Jam-124	India	Genetic diversity	2003 [40]
3	RAPD	Kalimpong-A, NB-1, NB-18, NB-4D2, NB-7, HU-204, Alps Yellow, Cevenese Yellow, Zebra (SL), S-36, Moria, Pure Mysore, C. niche, Nistari, Rong Daizo, Nistari (P), Suraput, Tamilnadu White, Hosa Mysore, Mysore Pincess	India	Genetic diversity	2005 [205]
4	SSR	Huiseluan, Sanmianbailuan, Duobanyueban, 3011B4Xin2, Heyuanlongjiao, Gansuzhong, Linliyuanxing, Sichunpuhuang, Ri7, Miancan, Changdejinhuang, Datuanyuan, Fa50B, Fa408, Bagdad, Huabasanwu, Suluanban, 306, 733Xin, Baozhongchang, Dazao, Dacao, Ri110, Ri120, Suluan7, Chunrichang, Hainanmianjian, Fang4, Qiongshanhainan, Nistari, P50	China	Genetic diversity	2005 [113]
5	SSR	Ri8, Ri9, Ri10, Ri11Xi, Ri12Xi, Ri13Xi, Ri13wu, Ri13qiao, J11wu, JA(-)Wu, Heizi, Jican, Tianlongqingbai, Yuzhong, European, Ou16wu, Ou17wu, Ou18, Ou19wu, AN525, Yi16, Diaosi1, Diaosi2, Fa50B, Fa403, N31Shihongwn, Xinnongoubai, Aizi1, Aizi2, Wusi1, Sulian1, Salisi1, Salisi15, Salisi22, Salisi24, East German201, East German 907, Luoni3, Luoni6, Luoni7, Luoni9, 317, 410, Ayag, Baohuang, Xiongyali, WeiluoA, Suluo42, Bailuo, Luobendihong, Luoshuyaohuang, Espana, Yintao, Turkey yellow cocoon, Weimala, Luosa, Mayila, Yuwufeibal, Asikeli, Morocco, 829, Huahe, EH1xin, T5, T9, T19, T20, Zhennong1, Chunlei, Zhong51, Zhong54, Zhong64, Zhong66, Deqing1, Deqing2, Yuhang7, Xinyi20, Dacao, Zhongnog29, Ping, Tai, 202, CB2, Tedaxingbai, Wuyuan11, CBI, Suzhi, 247, Cuxiong, Su17huang, 8212, Shennong1, Shennong2, Shennong3, Shennong5, Canmao1	China	Genetic diversity	2007 [84]
6	AFLP	Khorasan Lemon, Khorasan Orange, Khorasan Pink, P31, P103, P107	Iran	Genetic diversity	2007 [54]

Table 4 (continued)

SL. No	Marker type	Silkworm races/hybrid/stain	Country/region	Remarks	Year/[References]
7	ISSR	Huiseluan, Qiansanmian, 3011(D), Sanmianbai, Zhugui, Sanmianbailuan, Mengzi1, Gansu, Lu1, Ankang4, Lu10, Fengshui1, 43xin, 47xin, Xuyi1, Yuzhong, Taihe1, Ouzhou, Yanji, Xingshan2, Bagedate, Lanxi2, Ri7, Moluoge, Huaxin, Tianlongqingbai, Hua1, Hua5, Huabasanwu, 734, EH1xin, Fengnian, T9, Lanxi20, Yuhangerhua, Maisuoer, Dazao, Hainanmian, Su1, 3054, Su 3, Nistari	China	Genetic relationship	2007 [114]
8	ISSR	TMS-2, TMS-12, TMS-14, TMS-17, TMS-31, TMS-32, TMS-33, TMS-34, TMS-35, TMS-38, TMS-61, TMS-62, TMS-64, TMS-65, TMS-66, TMS-67, TMS-69, TMS-75, TMS-82, ODT	India	Genetic relationship	2008 [235]
9	RAPD, ISSR, RFLP-STS	Boropolu, Feng shong, R7042, European P, JZH (MO), NB_1, Tamilnadu white, M. princess, Rong Diazo, Nistari M, BL-23, MU-10, TMS-02, TMS-17, TMS-31, TMS-32, TMS-33, TMS-35	India	Genetic characterization	2008 [21][21]
10	RAPD	AB, IBV, B1, RG 90, Ac 29/T, Ac/T, H1, H2	Romania	Phylogenetic relationships	2009 [75]
11	ISSR	Kolar Gold, Kollegal Jawan, C.Nich, Sarupat, P4D3, PA12, Tamil Nadu White, Hosa Mysore, Mysore Princess, CB5, Nistari, Pure Mysore, NB4D2, SH6, YS3, CSR2, CSR3, CSR4, CSR6, CSR18, CSR19, NB7, NB18, CC1, CA2, PAM101, PAM111, P5, CSR2×4, CSR4×2	India	Genetic variability	2009 [193]
12	RAPD	Bursa Beyazı, Hatay sarısı, Alaca	Turkey	Genetic polymorphism	2009 [68]
13	RAPD	Alaca, Bursa Beyazı and Hatay Sarısı	Turkey	Molecular Analysis	2010 [8]
14	SSR	RMW_2, RMG_4, RBO_2, RBD_1, RMW_2 x RBO_2, RMG_4 x RBD_1	India	Nutrigenomic analysis	2010 [179]
15	SSR and mtDNA-SSCP	Tamilnadu White, Mysore Princess, Rong Daizo, BL-23, M2, Kollegal Jawan, Pure Mysore, Nistari (M), Nistari (P), Nistid (W), Moria, Nistari (D)	India	Genetic diversity	2010 [249]
16	SSR	N74, Usungrokeui, Usungjukei, Y54yu, N Hibakran, Ku27, Bagdad, Shansurian, Youlkukjam, IJPE, LT, Hojam, Kwasulpyung, Galwon, Tuk C60, C Hibakran, Sammyunhong, Urokbakran, Crimson, Q Hibakran, Hansunghukran, Hansungbanmun, HM, Kyunsakjuk, Hoknuwe, Y4, Hinode, Hansang 2ho, Qoichuk, Rok 191, AP, Z3, Je 1bakran, Jangsajang, YW, Damhukjam, O9C, Heukho, Ascori A, Hongbak, DY, Turkish, Oktaksoran, Rcp, J037, Eppanol, AT, Sandong sammyun, Jenam, Bm.W, Nd^H, Nd, Kore sammyun, Nd-s	Republic of Korea	Genetic relationship	2010 [107]
17	RAPD	C_{108}, NB_4D_2, Pure Mysore (PM), Nistari	India	Genetic Variability	2011 [216]

Table 4 (continued)

SL. No	Marker type	Silkworm races/hybrid/stain	Country/region	Remarks	Year/[References]
18	RAPD and ISSR	APM-1, BL-43, MU520, MW13, P4D3, Kolar Gold, M6M18, SK6 × SK1 × TW,TW × SK6 × SK1, Daizo, ZPN(SL), CB5, KW2, Nistari,	India	Genetic diversity	2011 [207]
19	SNP	Pure Mysore (P_1), CSR2 (P_2), PM × CSR2 F_1, PM × CSR2 F_2, (PM × CSR2) × PM, (PM × CSR2) × CSR2	India	Genetic analysis	2011 [204]
20	RAPD	S8, S8 × Ac 29/T, Ac 29/T × S8, Ac × B1, B1 × Ac, Hesa1 × Svila 2, B1 × Svila 2, B1 × Hesa 2,Vratza 35 × Svila 2	Romania	Phylogenetic relationships	2011 [76]
21	ISSR	Khorasan lemon, Harati lemon, Harati white, Harati yellow, Khorasan pink, Baghdadi, Guilan orange, Khorasan orange	Iran	Genetic diversity	2012 [176]
22	RAPD	AB, B75, S8, B1, AC29/T × S8, S8 × AC29/T, AC × B1, B1 × AC	Romania	Genetic diversity	2012 [253]
23	RAPD	ATR16, ATR29, BHR2, BHR3, B-37, CSR2, CSR17, CSR46, CSR47, CSR50, CSR51, D6(P), D6(P)N, NN6D, S-38, SK-3, SK-4, SK4C, Nistari, Cambodge	India	Genetic diversity	2013 [146]
24	SSR	CSR2, CSR50, CSR51, BHR3, SK4C, Pure Mysore, ND7, Nistari, Cambodge and L14	India	Genetic diversity	2014 [39]
25	RAPD	Sheikhi-I, Sheikhi-II, Tetrahybrid(P), SF-19, Kyorieshimpaku (P), Kyorieshimpaku (M), Shinki reyaku (M), NB4D2, SH-6, JD-6Sanish E2(M)	India	Genetic diversity	2015 [45]
26	RAPD	205MKD, 205MKD × 205PO, 205MKD × 206PO, 205MKD × C102, 205PO, 205PO × 206MKD, 205PO × J101, 206MKD, 206MKD × 205PO, 206MKD × C102, 206PO, C102, H × H, J101, C102 × 205MKD, C102 × 206MKD, C102 × J101, J101 × 205PO	Pakistan	Genetic diversity	2017 [25]
27	RAPD and ISSR	CSR_4, CSR_2, Pure Mysore, C. nichi	India	Genetic diversity	2017 [79]

Table 4 (continued)

SL. No	Marker type	Silkworm races/hybrid/stain	Country/region	Remarks	Year/[References]
28	SSR	Huiseluan, 3011(D), Sanmianbai, Sanmianbailuan, Duodueibanyueban, Xinlongjiao, 207, C2zhe, C110B, Yingwenpiban, Hehui Yinghan, 3011(Oxin3), 3011(B4xin2), 3011(5xin1), Hehuihuaba, Songhuaxingwu, Heyuanlongjiao, Fenshui No.1, Xiushui No.2, Suqian No.1, Ermao, Zhugui, Tangxisanmian, Zunyi No. 1, Zunyi No. 2, Zhengan No. 1, Yanhe No. 1, SanmianA, SanmianB, Sanguang, Lianyuen No. 1, Shangqiu No. 1, Mengzi No. 1, Luhuang1, 43xin, 47xin, 47qiao, Chaoxianzhong, Xiaofeng No. 17, Anji No.7, Yuhang11, Yuhang24, Yiwu No. 10, Xinchang No. 12, Linhai No. 39, Ninghai No. 20, Fenghua No. 7, Pixian No. 3, Tongshan No. 24, Xinyi No. 19, Sihong No. 15, Peixian No. 1, Fengxian No. 8, Yancheng No. 2, Mianyanghong, Xupuzhong, Changdejinhuang, Youxianzhong, Handanzhong, Datuanyuan, Jinhuang, Jinguang, Bilian, Zhong No. 11, Zhong No. 14, Balinghuang, Jiaxingyoucan, Yuhangbaipi, Jilisi, Longwangtang, Zhuji, Xiaobaiyuan, Xunba, Taihuyucan, Songhuacanji, Huayuan, Wulong 3030, 357, C17(zhuwu), 9008, 3042(18), Gong No. 2, Chuan35(1–4), Linchengzhong, Zhecan (hang), Zhenze, Lu108, Zhong20xi, Zhong21yu, Yanjizhong, Hangui(wu), CAzhe, Xugui(wu), Xingshan No. 1, Xingshan No. 2, Lanxi2	China	Genetic Fingerprint	2019 [174]

Artificial diet in sericulture

Among the several factors which contribute successful cocoon crop, good quality mulberry leaves alone contribute around 38.2 percent of the total share [144]. Moreover, quality of leaves also varies greatly in different growth stages of silkworm like young stage worms prefer leaves with high moisture content whereas insects at later stage require slightly coarser leaves. Apart from these there are some practical difficulties in feeding the silkworm particularly during rainy seasons because of wet leaves. Feeding of wet leaves may cause to build up extra humidity in the rearing bed which ultimately causing outbreak of several diseases. Moreover, shrinking of farmland for mulberry cultivation and labour shortage are some of the other reasons which compelled the scientists to work on the development of artificial diet. Therefore, the first ever attempt to rear silkworm on artificial diet was made way back in 1960 [74, 87] where entire rearing of the silkworm, *Bombyx mori* L., had been achieved on artificial diets which could not be so successful if imbalance of natural food nutrition for rearing silkworm due to crop failure would not have happended. There after several researchers have come up with different kinds of artificial diets [83] and advocated two different kinds of low-cost artificial diets for silkworm. One is these "Pellet diet", produced by using a twin-spindle extruder and can be fed to the silkworms by soaking with a suitable quantity of water just before use and another one is "Yuneri diet" that can be administered to the silkworms by mixing with hot water at around 80 °C without steaming. Trivedy et al. [228] developed a semi synthetic artificial diet'Seri Nutrid' upon evaluation on the performance of five multivoltine and six bivoltine strains, cocoons and associated parameters were found at par with that of mulberry leaf reared worms. Nair et al. [160] reported potentiality of chawki rearing for multivoltine silkworm exclusively on artificial diet to create breeding resource material for prospective hybrids. Further rearing of PM×CSR2 a popular silkworm cross breed on 'Seri Nutrid'

Table 5 Transgenesis in mulberry (*Morus* spp.)

Gene	Expression profile	References
*WAP21**	Cold tolerance	[229]
GUS	GUS incorporated into the protoplast through electroporation	[211]
Agrobacterium rizhgenes	Hairy roots	[163]
COR	Cold tolerance	[230]
GUS	GUS activity	[30]
AlaBlb	Salinity tolerance	[255]
OC	Insect resistance	[255]
SHN 1	Drought tolerance	[6]
GUS	Efficacy of different transformation technique	[3]
HVA1	Drought and salinity stress	[111]
bch1	High-temperature tolerances	[55]
bch	Drought and salinity stress	[104]
NHX	Drought and salinity stress	[104]
Osmotin	Drought and salinity stress	[58]
Hva1	drought, salinity and cold stress	[44]
HAL3a, dehydrin	Abiotic stress	[59]
nptII	GUS activity	[50]
bch1	Drought and salinity stress	[187]
AtSHN1	Abiotic stress	[192]
MmSK	drought stress	[116]
PCS1, PCS2	Heavy metal tolerance	[71]
MnACS1, MnACS3	salt and drought tolerances	[118]
MaCHS5, MaCHS6, MaCHS7, MaERF, MaDELLA, and MaJAZ	Identification and validation of reference genes	[52]
NPR1, NPR4	Abiotic stress resistance	[265]
Gtpase Era	Abiotic stress	[175]

recorded having significantly higher average cocoon weight, shell weight, and shell ratio as compared to sole feeding of mulberry [145]. In addition to mulberry leaves, feed supplements or fortification are also tried in silkworm but recent trend in use of probiotics has opened a new area for improvement of silkworm and cocoon production. Effect of probiotic and neutraceutical agent on growth, development, and commercial characteristics of *B. mori*, has recently been reported by many authors [23, 133, 202, 212, 252].

Non mulberry or wild silk culture is dominated by tasar silkworm *A. mylitta* which feeds primarily on *Terminalia arjuna* and *T. tomentosa* with many numbers of secondary and tertiary food plants. Unlike mulberry, rearing of tasar is totally outdoor and exposed to the natural vagaries causing a frequent crop loss. Therefore, efforts were also put forth for the development of artificial feed for tasar silkworm. Continuous research efforts finally resulted in an effective formulation of artificial feed named 'Tasar Amrit', especially for rearing of juvenile silkworm. Field studies with 'Tasar Amrit' as feed for tasar silkworm have shown a great promise with higher survival rate and effective frequency of rearing compared to complete outdoor rearing [190]. The performance of 'Tasar Amrit' was further substantiated by Kumar et al. [110] where they achieved higher survival of young age worms (88.82%) and effective rate of rearing (46.95%) in comparison to feeding fresh leaves in indoor rearing and complete outdoor rearing of tasar silkworm.

Application of biotechnology in silkworm disease management

Diseases of silkworm have always been a matter of great concern for sericulture and silk production. Silkworms are known to be affected by number of diseases namely grasserie, flacherie, muscardine and pebrine caused by virus, bacteria, fungi and microsporidia, resulting frequent crop failure in practical situations [20, 22, 89]. Around 15 to 20 kg of cocoons per 40,000 silkworm larvae are estimated to be lost due to silkworm diseases in India [196]. The most common methodologies used for containment of silkworm diseases include preventive measures, microscopy, in vivo assays like enzyme-linked immunosorbent assay (ELISA) [232], DNA hybridization [18], colloidal textile dye-based dipstick immunoassay [161], protein-A linked monoclonal

antibody latex agglutination test (PALMAL) [197] and viral DNA transfection [131] are some of the techniques that were developed with respect to silkworm diseases management. But early and rapid detection of pathogens such as nuclear polyhedrosis virus, densonucleosis virus, infectious flatcherie virus, *Nosema bombycis* in silkworm will help in the rejection of crop and replacement with new one to prevent further spread of diseases. Therefore, use of modern biotechnological tools for rapid detection and management of silkworm diseases are gaining momentum now a days. A series of different PCR based rapid diagnostic method particularly with reference to rapid detection of microsporidian and viral diseases of silkworm have been reported in recent times [1, 21, 90, 106, 186, 219, 254]. Use of LAMP (Loop Mediated Isothermal Amplification) for rapid detection of pebrine and engineering silkworm for baculovirus resistance through RNA Interference (RNAi) technology are some of the emerging areas of biotechnological innervations in management of silkworm diseases [53, 69, 95, 209, 233].

Silkworm as bioreactor for expression of foreign protein

Isolation, cloning, characterisation, and expression of a gene of interest have become possible because of the improvement in the modern techniques of genetic engineering. The expression of pharmaceutically important proteins, namely cell/viral surface proteins, membrane proteins, and guanine nucleotide-binding protein (G protein) coupled receptors in silkworm larvae or cocoons has opened a new area in the silkworm biotechnology. Further, use of silkworm larvae rather than cultured cell lines as recipient for expression of foreign protein in *Bombyx mori* nucleo polyhedrovirus (BmNPV) has become an effective alternative for the large-scale synthesis of commercially important biomolecules**.** Maeda et al. [128] first reported the production of human α-interferon (IFN-α) in the hemolymph of silkworm larvae using BmNPV expression system. Likewise, in the recent past, a lot of reports were surfaced on the production of different proteins or important biomolecules of commercial importance using silkworm as a model. Production of recombinant erythropoietin (rEpo) in silkworm larvae using Bm NPV expression system has been attempted successfully in recent times [100]. To overcome the problem of degradation of foreign protein in BmNPV system, cysteine protease depleted BmNPV was also tried for isolation of bovine interferon-τ from silkworm larvae [154]. While Muneta et al. [149] purified 500 µg of bovine interleukin-21 (IL-21) from 30 ml of hemolymph using a hybrid baculovirus. Construction of a recombinant baculovirus, containing a gene of interest requires transfection of cell culture with the baculovirus and the transfer vector which is time consuming and cumbersome one. Therefore, development of a recombinant baculovirus vector (Bacmid) that can replicate in *E. coli* as a large plasmid is known as Bac-to-Bac baculovirus expression system has come into picture [124]. Bac-to-Bac system has addressed many issues related to expression of foreign protein in BmNPV expression system. Motohashi et al. [147] while constructing a BmNPV bacmid established the Bac-to-Bac system using BmNPV. GFPuv was expressed only by the injection of BmNPV bacmid DNA into silkworm larvae and pupae. Production of capsid proteins for foot-and-mouth disease virus (FMDV) in the hemolymph of silkworm larvae and vaccination of 30 fold diluted hemolymph for protection of cattle against FMDV [115] is another significant attempt in this regard. Expression and purification of human (pro) renin receptor (hPRR) and its complex with human prorenin were also tried in silkworm larvae, with the same expression level as some secretory proteins [65, 66]. In the country like India, where art of rearing of silkworm is as old as our heritage, holds a lot of promise and opportunities to utilize it as biofactory for production of important proteins, vaccines, and biomaterials.

Silk based biomaterials

Fabrication and use of silk based biomaterials like sature, silkbased hydrogels, cosmetics, scaffolding matrix, 2D films, nanofibrous mat have drawn considerable attention to the scientific fraternity in recent times. India is bestowed with all four kinds of silk which provide immense opportunity to explore use of silk for non textile purposes, particularly in production and application of silk protein based biomaterials. Padamwar et al. [168] while studying in vivo effect of sericin on human skin reported prevention of transepidermal water loss, responsible for skin dryness confirming the moisturizing effect of sericin. The study indicated action of sericin in increasing the level of hydroxyproline and hydration of the epidermal cells. Recombinant silk protein/ spider silk has also been used in many avenues such as commercial, military and clinical research. Spider silk has been used in prepration of bullet proof jacket and utilized for its wound healing property, especially in surgery as its biodegradable. Role of silk protein in stimulating the migration, proliferation, and production of collagen provided evidence on the healing properties of sericin, [13–15]. Aramwit et al. [14] also highlighted the importance of amino acid methionine of sericin in collagen synthesis, essential in the healing process. In vivo skin wound healing properties of fibroin films [210] and fibroin-alginate sponges [183] have also been found to be better compared to other clinically used materials. Mixing of silk fibroin with human hair derived Keratin has also been examined for wound healing application in skin tissue engineering [126]. Another encouraging attempt of present times is the fabrication of tasar silk fibroin based corneal films [81]. Application of tasar silk fibroin as cardiac patch

because of its fibronectine like properties has also been documented [169]. Recent report suggests that silk and silk fibroin films with suitable mechanical properties may be useful as artificial blood vessels as well as for vascular graft [28, 115, 117, 127, 194]. Further, manufacturing of 2D matrices from silk gland fibroins of muga silk and tasar silk along with cocoon fibroin of mulberry silk have been studied recently [99]. In another attempt fabrication of 3D scaffolds under single and double-seeded conditions using tasar silk fibroin extracted from silk gland showed improvement of oestrogenic potentials of the scaffolds [130]. Muga silk fibroin also has got tremendous potentiality to act as an appropriate material for fabrication of functional scaffolds to support cartilage tissue engineering [29]. Orthopaedic and dental surgeries are very much prone to loosening, inflammation, bacterial infections. Tasar silk fibroin nanoparticles coupled with gentamycin supports better in osteoblast adhesion when compared to bare Ti surface [199]. Therefore, use of silk protein for synthesising novel biomaterials may open a new area in sericulture.

Waste utilization

Apart from silk, several other by-products of economic importance are being generated by silk industry including waste mulberry shoots, silkworm moth, unused silkworm pupae, tasar peduncle, cut cocoons. Among the several kinds of waste generated by the silk industry unused silkworm pupae known to contribute maximum share. The pupae, which are obtained after reeling of silk threads from cocoons, are generally thrown away though they are very rich in amino acids, oil, carbohydrate and minerals. According to an estimate approximately 40,000 MT of silkworm pupae is produced through sericulture, per annum in our country. Traditional practice of drying and disposal of silkworm pupae cause environmental pollution besides loosing important nutrients in them. But this huge waste of silk industry holds a great promise to convert them into meaningful wealth. Consuming 100 gm of (dry weight) of silkworm pupae may substitute ingestion of approximately 56 gm of protein, which satisfies the recommended dietary allowance of protein for an adult [136]. Silkworm pupae protein is considered to be a newly available source that contains all the amino acids needed by the human body. Recently, it has been documented that silkworm pupae have excellent antioxidant potential to scavenge free radicals with good antityrosinase activity [61, 62]. Proximate analysis of pupa showed that it contains 55–60% protein, 25–30% lipid, 4.96% fiber, and other substances like, hormones, trace elements and vitamins, thus indicating the pupa as a good protein source for various purposes [120, 121, 180, 269]. The silkworm pupal oil is highly enriched with α-linolenic acid which has got many uses in oleo chemical and food processing industries. Chitin and chitosan, the two important products can be obtained from silkworm skin and pupae and both these materials are known to possess antimicrobial properties with capacity of biodegradability and biocompatibility. In India, the indigenous population of north east India uses different insects as food and among them eri and muga silkworms are two important insects which are most popular as traditional food. The consumption of pupa is maximum for eri (87%) followed by muga (57.4%) and mulberry (24.6%) [140]. Additionally, silkworm pupae are known to be an important food for poultry and fish industries along with its application as base materials for production of highly valued mushrooms [37].

Waste silkworm pupae hold a great promise in many advanced fields including cosmetics, animal nutrition and pharmaceuticals with commercial importance. However, no comprehensive work has been carried out on the utilization of high potential secondary wastes of silk industry which can further substantiate the profit for the silk industry. This excellent source of good quality protein offers huge potential for nutritional and economic benefits to marginal sericulture farmers if proper utilization is made from these by products of the silk industry.

Conclusion

Sericulture implies rearing of silkworm for production of silk and ultimately its usage for textile and garment. Time has come to diversify it to make sericulture more sustainable, lucrative, and remunerative one. Therefore, in the present day, focus has started shifting on the use of silk for non textile purposes. Inclusive development of sericulture is only possible if improvement in silk productivity and application of silk for non textile purposes grows together side by side. Modern biotechnological advancement has assumed greater importance in the development of sericulture and its diversification. The science of biotechnology and its application particularly in the area of identifying new genes, molecular markers, development of diseases resistant breed and feed, synthesis of biologically important molecules, formulation of artificial diet and conversion of seri waste into wealth may take sericulture into a new height. Further application of silk protein biomedical science through modern techniques has proved to be one of the new and promising area of applying sericulture other than non textile purposes. The increased approaches of biotechnological applications can certainly revolutionise the entire seri industry besides playing its role in maintaining ecological balance. The present paper, therefore, is a comprehensive document where an attempt to accumulate different reports and research findings on thrust areas of sericulture like molecular markers, transgenesis, embryogenesis, synthesis of biomolecules, chromosome

engineering, use of artificial feed and waste management issues have been discussed which will prove to be useful for further development of the silk industry in coming years.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13237-021-00355-2.

Acknowledgements The authors are thankful to Raiganj University and University of Kalyani, West Bengal, India for providing instrumentation and library facilities.

Authors' contributions KA provided the conception and design of the study, drafting the article; RVS: supplied the acquisition of data, drafting of manuscript; TC: supplied the acquisition of data, drafting of manuscript; AB: supplied the acquisition of data; PDG: supplied the acquisition of data; SS: provided the critically revised article with important intellectual content and gave final approval of this version to be submitted.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. Abulyazid I, Elshafei A, Mousa S, El-Said E, Taha R. Molecular diagnosis for the silkworm *Bombyx mori* L. viral and bacterial diseases in the irradiated and non irradiated individuals. Isotope Radiat Res. 2007;39:545–66.
2. Agarwal S. Genetic transformation and plant regeneration studies in *Morus alba* L. Doctoral thesis. Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, India. 2002.
3. Agarwal S, Kanwar K. Comparison of genetic transformation in *Morus alba* L. via different regeneration systems. Plant Cell Rep. 2007;26:177–85.
4. Agarwal S, Kanwar K, Sharma DR. Factors affecting secondary somatic embryogenesis and embryo maturation in *Morus alba* L. Scientia Hortic. 2004;102:359–68.
5. Aggarwal RK, Udaykumar D, Hender PS, Sarkar A, Singh L. Isolation and characterization of six novel microsatellite markers for mulberry (*Morus indica*). Mol Ecol Notes. 2004;4:477–9.
6. Aharoni A, Dixit S, Jetter R, Van Thoenes, AG, Pereira A. The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alterscuticle properties, and confers drought tolerance when over expressed in Arabidopsis. Plant Cell. 2004;16:2463–80.
7. Ahmad P, Sharma S, Srivastava PS. *In vitro* selection of $NaHCO_3$ tolerant cultivars of *Morus alba* (Local and Sujanpur) in response to morphological and biochemical parameters. Hortic Sci. 2007;34:114–22.
8. Akkir DE, Yildiran FAB, Çakir S. Molecular analysis of three local silkworm breeds (Alaca, Bursa Beyazı and Hatay Sarısı) by RAPD-PCR and SDS-PAGE methods. Kafkas Univ Vet Fak Derg. 2010;16:S265–9.
9. Akram M, Aftab F. Efficient micropropagation and rooting of king white mulberry (*Morus macroura* Miq.) var. laevigata from nodal explants of mature tree. Pak J Bot. 2012;44:285–9.
10. Anil Kumar HV, Muralidhar TS, Munirajappa R. RAPD analysis of EMS mutagenised mulberry genotype RFS135. Schol J Biotechnol. 2012;1:1–7.
11. Anis M, Faisal M, Singh SK. Micropropagation of mulberry (*Morus alba* L.) through in vitro culture of shoot tip and nodal explants. Plant Tissue Cult. 2003;13:47–51.
12. Anuradha JH, Vijayan K, Nair CV, Manjula A. A novel and efficient protocol for the isolation of genomic DNA from mulberry (*Morus* L.). Emir J Food Agric. 2013;25:124–31.
13. Aramwit P, Sangcakul A. The effects of sericin cream on wound healing in rats. Biosci Biotechnol Biochem. 2007;71:2473–7.
14. Aramwit P, Kanokpanont S, De-Eknamkul W, Kamei K, Srichana T. The effect of sericin with variable amino-acid content from different silk strains on the production of collagen and nitric oxide. J Biomater Sci Polym Ed. 2009;20:1295–306.
15. Aramwit P, Kanokpanont S, Nakpheng T, Srichana T. The effect of sericin from various extraction methods on cell viability and collagen production. Int J Mol Sci. 2010;11:2200–11.
16. Arora V, Ghosh MK, Gangopadhyay G. SSR markers for assessing the hybrid nature of two high yielding mulberry varieties. Int J Genet Eng Biotechnol. 2014;52:191–6.
17. Arora V, Ghosh MK, Pal S, Gangopadhyay G. Allele specific CAPS marker development and characterization of chalcone synthase gene in Indian mulberry (*Morus* spp., family Moraceae). PLoS ONE. 2017;12:e0179189.
18. Attathom T, Attathom S, Kumpratueang S, Audtho M. Early detection of Grasserie disease of silkworm, *Bombyx mori* by DNA probe. In: Proceeding of 32nd Kasetsart University annual conference: plant science, Kasetsart University, Bangkok, Thailand; 1994. pp. 257–271.
19. Awasthi AK, Nagaraja GM, Naik GV, Kanginakudru S, Thangavelu K, Nagaraju J. Genetic diversity in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. BMC Genet. 2004;5:1–9.
20. Awasthi AK, Pradeep AR, Srivastava PP, Vijayan K, Kumar V, Urs SR. PCR detection of densonucleosis virus isolates in silkworm (*Bombyx mori*) from India and their nucleotide variability. Indian J Biotechnol. 2008;7:56–60.
21. Awasthi AK, Kar PK, Srivastava PP, Nidhi R, Vijayan K, Pradeep AR, Urs SR. Molecular evaluation of bivoltine, polyvoltine and mutant silkworm (*Bombyx mori* L.) with RAPD, ISSR and RFLP-STS markers. Indian J Biotechnol. 2008;7:188–94.
22. Babu KR, Ramakrishna S, Reddy YHK, Lakshmi G, Naidu NV, Basha SS, Bhaskar M. Metabolic alterations and molecular mechanism in silkworm larvae during viral infection: a review. Afr J Biotechnol. 2009;8:899–907.
23. Bai PKKS, Bai MR. Studies on the effect of a probiotic and a neutraceutical agent on growth, development and commercial characteristics of silkworm, *Bombyx mori*. Indian J Seric. 2012;51:37–42.
24. Bajaj YPS. Biotechnology in agriculture and foresty, vol. 2. Berlin: Springer Verlag; 1986.
25. Bajwa GA, Ahmed N, Shah SH, Adnan M. Genetic diversity analysis of mulberry silkworm (*Bombyx mori*) strains using RAPD markers. J Anim Plant Sci. 2017;27:575–81.
26. Balakrishnan V, Latha MR, Ravindran KC, Robinson JP. Clonal propagation of *Morus alba* L. through nodal and axillary bud explants. Bot Res Int. 2009;2:42–9.
27. Banerjee R, Chattopadhyay S, Saha AK. Genetic diversity and relationship of mulberry genotypes revealed by RAPD and ISSR markers. J Crop Improv. 2016;30:478–92.
28. Bhardwaj N, Rajkhowa R, Wang X, Devi D. Milled non-mulberry silk fibroin microparticles as biomaterial for biomedical applications. Int J Biol Macromol. 2015;81:31–40.
29. Bhardwaj N, Singh YP, Devi D, Kandimalla R, Kotoky J, Mandal BB. Potential of silk fibroin/chondrocyte constructs of muga silkworm *Antheraea assamensis* for cartilage tissue engineering. J Mater Chem B. 2016;4:3670–84.

30. Bhatnagar S, Khurana P. *Agrobacterium tumefaciens*-mediated transformation of Indian mulberry, *Morus indica* cv. K-2: a time phased screening strategy. Plant Cell Rep. 2003;21:669–75.
31. Bhatnagar S, Kapur A, Khurana P. TDZ mediated differentiation in commercially valuable Indian mulberry, *Morus indica* cultivars K2 and DD. Plant Biotechnol. 2001;18:61–5.
32. Bhatnagar S, Kapur A, Khurana P. Evaluation of parameters for high efficiency gene transfer via particle bombardment in Indian mulberry. Indian J Exp Biol. 2002;40:1387–93.
33. Bhattacharya E, Ranade SA. Molecular distinction amongst varieties of Mulberry using RAPD and DAMD profiles. BMC Plant Biol. 2001;1:1–8.
34. Bhau BS, Wakhlu AA. Effect of genotype, explant type and growth regulators on organogenesis in *Morus alba*. Plant Cell Tiss Organ Cult. 2001;66:25–9.
35. Bhau BS, Wakhlu AK. Rapid micropropagation of five cultivars of mulberry. Biol Plant. 2003;46:349–55.
36. Biondi S, Thorpe TA. Clonal propagation of forest tree species. In: Rao A, editor. Proceedings of COSTED symposium-"Tissue culture of economically important plants", COSTED and Asian Network of Biological Sciences, Singapore; 1982. p. 197–204.
37. Buhroo ZI, Bhat MA, Kamili AS, Ganai NA, Bali GK, Khan IL, Aziz A. Trends in development and utilization of sericulture resources for diversification and value addition. J Entomol Zool Stud. 2018;6:601–15.
38. Chakraborty S, Muthulakshmi M, Vardhini D, Jayprakash P, Naagraju J, Arunkumar KP. Genetic analysis of Indian tasar silkmoth (*Antheraea mylitta*) populations. Sci Rep. 2015;5:15728. https://doi.org/10.1038/srep15728.
39. Chandrakanth N, Moorthy SM, Dayananda AP, Ashwath SK, Kumar V, Bindroo BB. Evaluation of genetic diversity in silkworm (*Bombyx mori* L.) strains using microsatellite markers. Int J Biotechnol Allied Fields. 2014;2:73–93.
40. Chatterjee SN, Pradeep AR. Molecular markers (RAPD) associated with growth, yield, and origin of the silkworm, *Bombyx mori* L, in India. Russ J Genet. 2003;39:1365–77.
41. Chatterjee SN, Vijayan K, Roy GC, Nair CV. ISSR profiling of genetic variability in the ecotypes of *Antheraea mylitta* Drury, the tropical tasar silkworm. Russ J Genet. 2004;40:152–9.
42. Chatterjee SN, Nagaraja GM, Srivastava PP, Naik G. Morphological and molecular variation of *Morus laevigata* in India. Genetica. 2004;39:1612–24.
43. Chattopadhyay S, Doss SG, Halder S, Ali AK, Bajpai AK. Comparative micropropagation efficiency of diploid and triploid mulberry (*Morus alba* cv. S1) from axillary bud explants. Afr J Biotechnol. 2011;10:18153–9.
44. Checker VG, Chhibbar AK, Khurana P. Stress-inducible expression of barley *Hva1* gene in transgenic mulberry displays enhanced tolerance against drought, salinity and cold stress. Transgenic Res. 2012;21:939–57.
45. Chikkaswamy BK, Paramanik RC. Molecular distinction of silkworm varieties using RAPD molecular marker. Int J Curr Res Acad Rev. 2015;3:199–208.
46. Chikkaswamy BK, Prasad MP. Evaluation of genetic diversity and relationships in mulberry varieties using RAPD and ISSR molecular markers. Int J Mol Biol. 2012;3:2–7.
47. Chikkaswamy BK, Paramanik RC, Debnath A, Sadana MS. Evaluation of genetic diversity in mulberry varieties using molecular markers. Nat Sci. 2012;10:45–60.
48. Chitra DSV, Padmaja G. Seasonal influence on axillary bud sprouting and micropropagation of elite cultivars of mulberry. Sci Hortic. 2002;92:55–68.
49. Chitra DSV, Padmaja G. Shoot regeneration via direct organogenesis from in vitro derived leaves of mulberry using thidiazuron and 6-benzylaminopurine. Sci Hortic. 2005;106:593–602.
50. Chitra DSV, Chinthapalli B, Padmaja G. Efficient regeneration system for genetic transformation of mulberry (*Morus indica* L. Cultivar S-36) using in vitro derived shoot meristems. Am J Plant Sci. 2014;5:1–6.
51. Choudhary R, Chaudhury R, Malik SK, Kumar S, Pal D. Genetic stability of mulberry germplasm after cryopreservation by two-step freezing technique. Afr J Biotechnol. 2013;12:5983–93.
52. Dai F, Zhao X, Tang C, Wang Z, Kuang Z, Li Z, Huang J, Luo G. Identification and validation of reference genes for qRT-PCR analysis in mulberry (*Morus alba* L.). PLoS ONE. 2018;13:e0194129.
53. Dai W, Qi J, Chen H, Zhang Z, Shang R, Zhang Y, Tang S, Shen Z. Rapid and sensitive detection of *Nosema bombycis* using loop-mediated isothermal amplification and colorimetric nanogold. Sci Asia. 2019;45:268–74.
54. Dalirsefat SB, Mirhoseini SZ. Assessing genetic diversity in Iranian native silkworm (*Bombyx mori* L.) strains and Japanese commercial lines using AFLP markers. Iran J Biotechnol. 2007;5:25–33.
55. Das M. Screening and genetic manipulation of mulberry for abiotic stress tolerance. PhD thesis, Delhi University. 2009.
56. Das BC, Krishnaswami S. Some observations on inter-specific hybridization in mulberry. Indian J Seric. 1965;4:1–4.
57. Das BC. Mulberry taxonomy, cytogenetics and breeding. National seminar on silk research and development, Bangalore, India, 10–13 March 1983.
58. Das M, Chauhan H, Chhibbar A, Rizwanul Haq QM, Khurana P. High-efficiency transformation and selective tolerance against biotic and abiotic stress in mulberry, *Morus indica* cv. K2, by constitutive and inducible expression of tobacco osmotin. Transgenic Res. 2011;20:231–46.
59. Das M, Tetoriya M, Haq QMR, Khurana P. Screening and expression analysis of *hal3a, dehydrin* and *nhx1* in ten genotypes of mulberry for abiotic stress tolerance. Sericologia. 2013;53:1–10.
60. Datta RK. Guidelines for bivoltine rearing. Central Silk Board, Bangalore, India;1992. p. 24.
61. Deori M, Devi D, Devi R. Nutrient composition and antioxidant activities of Muga and Eri silkworm pupae. Int J Sci Nat. 2014;5:636–40.
62. Deori M, Boruah DC, Devi D, Devi R. Antioxidant and antigenotoxic effects of pupae of the muga silkworm *Antheraea assamensis*. Food Biosci. 2014;5:108–14.
63. Desai S, Desai P, Mankad M, Patel A, Patil G, Narayanan S. Development of micropropagation protocol for *Morus nigra* L. (black mulberry) through axillary buds. Int J Chem Stud. 2018;6:585–9.
64. Doira H, Fujii H, Kawaguchi Y, Kihara H, Banno Y. Genetical stocks and mutations of *Bombyx mori*: important genetic resources. Inst Genet Resour. Fac Agr Kyushu Univ Fukuoka. 1992. p. 74.
65. Du D, Kato T, Nurun Nabi AHM, Suzuki F, Park EY. Expression of functional human (pro)renin receptor in silkworm (*Bombyx mori*) larvae using BmMNPV bacmid. Biotechnol Appl Biochem. 2008;49:195–202.
66. Du D, Kato T, Suzuki F, Park EY. Binding affinity of full length and extracellular domains of recombinant human (Pro)renin receptor to human renin when expressed in the fat body and hemolymph of silkworm larvae. J Biosci Bioeng. 2009;108:304–9.
67. Duarte WN, Zanello CA, Cardoso JC. Efficient and easy micropropagation of *Morus nigra* and the influence of natural light on its acclimatization. Adv Hortic Sci. 2019;33:433–9.
68. Eroglu D, Arica SC. Molecular genetic analysis of three Turkish local silkworm breeds (Bursa Beyazı, Alaca and Hatay Sarısı) by RAPD-PCR method. J Appl Biol Sci. 2009;3:17–20.

69. Esvaran VG, Gupta T, Mohanasundaram A, Ponnuvel KM. Development of isothermal amplification assay for detection of *Nosema bombycis* infection in silkworm *Bombyx mori* targeting polar tube protein 1 gene. Invertebr Surviv. 2018;15:352–61.
70. Ezeonu CS, Richard Tagbo R, Anike EN, Obinna A, Oje A, Ikechukwu NEO. Biotechnological tools for environmental sustainability: prospects and challenges for environments in Nigeria—a standard review. Biotechnol Res Int. 2012. https://doi.org/10.1155/2012/450802.
71. Fan W, Liu C, Cao B, Qin M, Long D, Xiang Z, Zhao A. Genome-wide identification and characterization of four gene families putatively involved in cadmium uptake, translocation and sequestration in mulberry. Front Plant Sci. 2018;9:879.
72. Fang S-M, Zhou Q-Z, Yu Q-Y, Zhang Z. Genetic and genomic analysis for cocoon yield traits in silkworm. Sci Rep. 2020;10:5682. https://doi.org/10.1038/s41598-020-62507-9.
73. Feng LC, Guangwei Y, Maode Y, Yifu K, Chenjun J, Zhonghuai Y. Studies on the genetic identities and relationships of mulberry cultivated species (*Morus* L.) by a random amplified polymorphic DNA assay. Acta Sericol Sin. 1996;22:135–9.
74. Fukuda T, Suto M, Higuchi Y. Silkworm raising on artificial food. Nature. 1960;187:669–70.
75. Furdui E, Mărghitaş LA, Dezmirean D, Paşca I, Coroian C. RAPD analysis of romanian silkworm genetic biodiversity (races X hybrids). Bull UASVM Anim Sci Biotechnol. 2009;66:430–4.
76. Furdui EM, Marghita LA, Dezmirean D, Pop IF, Coroian C, Pasca I. Genetic phylogeny and diversity of some Romanian silkworms based on RAPD technique. J Anim Sci Biotechnol. 2011;44:204–8.
77. Gogoi G, Borua PK, Al-Khayri JM. Improved micropropagation and in vitro fruiting of *Morus indica* L. (K-2 cultivar). J Genet Eng Biotechnol. 2017;15:249–56.
78. Guruprasad Krishnan RR, Dandin SB, Naik VG. Groupwise sampling: a strategy to sample core entries from RAPD marker data with application to mulberry. Trees. 2014;28:723–31.
79. Haghighi MT, Jagadeesh Kumar TS. Genetic divergence and allelic-specificity in relation to expression of voltinism in silkworm using ISSR and RAPD fingerprinting. Russ J Genet. 2017;53:267–74.
80. Hawramee AOK, Aziz RR, Hassan DA. Propagation of white mulberry *Morus alba* L. fruitless cultivar using different cutting times and IBA. IOP Conf Ser Earth Environ Sci. 2019;388:012069.
81. Hazra S, Nandi S, Naskar D, Guha R, Chowdhury S, Pradhan N, Kundu SC, Konar A. Non-mulberry silk fibroin biomaterial for corneal regeneration. Sci Rep. 2016;6:21840.
82. He N, Zhang C, Qi X, Zhao S, Tao Y, Yang G, Lee TH, Wang X, Cai Q, Li D, Lu M, Liao S, Luo G, He R, Tan X, Xu Y, Li T, Zhao A, Jia L, Fu Q, Zeng Q, Gao C, Ma B, Liang J, Wang X, Shang J, Song P, Wu H, Fan L, Wang Q, Shuai Q, Zhu J, Wei C, Zhu-Salzman K, Jin D, Wang J, Liu T, Yu M, Tang C, Wang Z, Dai F, Chen J, Liu Y, Zhao S, Lin T, Zhang S, Wang J, Wang J, Yang H, Yang G, Wang J, Paterson AH, Xia Q, Ji D, Xiang Z. Draft genome sequence of the mulberry tree *Morus notabilis*. Nat Commun. 2013;4:2445. https://doi.org/10.1038/ncomms3445.
83. Hiroshi S, Hiro-aki Y. Low-cost artificial diets for polyphagous silkworms. Jpn Agric Res Q. 1994;28:262–7.
84. Hou CX, Li MW, Zhang YH, Qian HY, Sun PJ, Xu AY, Miao XX, Guo QH, Xiang H, Huang YP. Analysis of SSR fingerprints in introduced silkworm germplasm resources. Agric Sci China. 2007;6:620–7.
85. Huang R-Z, Yan X-P, Li J, Zhang X-W. AFLP fingerprint analysis for 10 mulberry cultivars in Hunan province. Sci Seric. 2009;35:837–41.
86. Ipek M, Pirlak L, Kafkas S. Molecular characterization of mulberry (*Morus* spp.) genotypes via RAPD and ISSR. J Sci Food Agric. 2012;92:1633–7.
87. Ito T, Tanaka M. Rearing of the silkworm on an artificial diet and the segregation of pentamolters. J Seric Sci. 1960;29:191–6.
88. Ji T, Shuang F, Aizhen Y, Ning D, Yueping L. the primary study of mulberry rapid propagation by culture of side bud tissue. Chin Agric Sci Bull. 2008; Article No. 47.
89. Jiang L, Xia QY. The progress and future of enhancing antiviral capacity by transgenic technology in the silkworm *Bombyx mori*. Insect Biochem Mol Biol. 2014;48:1–7.
90. Joshi RP, Raja IA. PCR based conformation of viral flacherie in the silkworm, *Bombyx mori*. Biosci Biotechnol Res Commun. 2015;8:189–92.
91. Kadono-Okuda K, Kosegawa E, Mase K, Hara W. Linkage analysis of maternal EST cDNA clones covering all twenty-eight chromosomes in the silkworm, *Bombyx mori*. Insect Mol Biol. 2002;11:443–51.
92. Kafkas S, Ozgen M, Dogan Y, Ozgen B, Ercisli S, Serce S. Molecular characterization of mulberry accessions in Turkey by AFLP markers. J Am Soc Hortic Sci. 2008;133:593–7.
93. Kala P, Zargar SM, Bali RK, Gupta N, Salgotra RK, Koul A. Assessment of genetic diversity in mulberry using morphological and molecular markers. Electron J Plant Breed. 2016;7:94–103.
94. Kalpana D, Choi SH, Choi TK, Senthil K, Lee YS. Assessment of genetic diversity among varieties of mulberry using RAPD and ISSR fingerprinting. Sci Hortic. 2012;134:79–87.
95. Kampliw S, Monthatong M. Loop mediated isothermal amplification (LAMP) for *Nosema bombycis* diagnosis by small subunit ribosomal RNA (SSU rRNA) gene. Indian J Agric Res. 2019;53:447–52.
96. Kapur A, Bhatnagar S, Khurana P. Efficient regeneration from mature leaf explants of Indian mulberry via organogenesis. Sericologia. 2001;41:207–14.
97. Kar PK, Vijayan K, Mohandas TK, Nair CV, Saratchandra B, Thangavelu K. Genetic variability and genetic structure of wild and semi-domestic populations of tasar silkworm (*Antheraea mylitta*) ecoraces Daba as revealed through ISSR markers. Genetica. 2005;125:173–83.
98. Kar P, Srivastava PP, Awasthi AK, Urs SR. Genetic variability and association of ISSR markers with some biochemical traits in mulberry (*Morus* spp.) genetic resources available in India. Tree Genet Genomes. 2008;4:75–83.
99. Kar S, Talukdar S, Pal S, Nayak S, Paranjape P, Kundu S. Silk gland fibroin from Indian muga silkworm *Antheraea assama* as potential biomaterial. Tissue Eng Regen Med. 2013;10:200–10.
100. Kari K, David M. Expression of human erythropoietin protein using a Baculovirus. Int J Recent Sci Res. 2015;6:3515–9.
101. Karnosky DF. Potential for forest tree improvement via tissue culture. Bioscience. 1981;31:114–20.
102. Kashyap S, Sharma S. In vitro selection of salt tolerant *Morus alba* and its field performance with bioinoculants. Hortic Sci. 2006;33:77–86.
103. Kavyashree R, Gayatri MC, Revanasiddaiah HM. A repeatable protocol for the production of gynogenic haploid plants in mulberry. Sericologia. 2001;41:517–21.
104. Khurana P. Mulberry genomics for crop improvement. In: Saratchandra B, Singh RN, Vijayan K, editors. Workshop on recent advances in sericulture re-search. Bangalore: Central Silk Board; 2010. p. 35.
105. Khurana P, Checker VG. The advent of genomics in mulberry and perspectives for productivity enhancement. Plant Cell Rep. 2011;30:825–38.
106. Khyade VB, Tyagi BK. Detection of grasserie virus, BmNPV in the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM

x CSR2) through polymerase chain reaction. Int J Curr Microbiol Appl Sci. 2017;6:13–23.
107. Kim KY, Kang PD, Lee KG, Oh HK, Kim MJ, Kim K, Park SW, Lee SJ, Jin BR, Kim I. Microsatellite analysis of the silkworm strains (*Bombyx mori*): high variability and potential markers for strain identification. Genes Genom. 2010;32:532–43.
108. Kojima K, Kuwana Y, Sezutsu H, Kobayashi I, Uchino K, Tamura T, Tamada Y. A new method for the modification of fibroin heavy chain protein in the transgenic silkworm. Biosci Biotechnol Biochem. 2007;71:2943–51.
109. Krishnan RR, Naik VG, Ramesh SR, Qadri SMH. Microsatellite marker analysis reveals the events of the introduction and spread of cultivated mulberry in the Indian subcontinent. Plant Genet Resour. 2013;12:129–39.
110. Kumar D, Pandey JP, Sinha AK, Salaj S, Mishra PK, Prasad BC. Evaluation of novel tasar silkworm feed for *Antheraea mylitta*: its impact on rearing, cocoon trait and biomolecular profile. Am J Biochem Mol Biol. 2013;3:167–74.
111. Lal S, Gulyani V, Khurana P. Overexpression of *HVA1* gene from barley generates tolerance to salinity and water stress in transgenic mulberry (*Morus indica*). Transgenic Res. 2008;17:651–63.
112. Lalitha N, Kih S, Banerjee R, Chattopadhya S, Saha AK, Bindroo BB. High frequency multiple shoot induction and in vitro regeneration of mulberry (*Morus indica* L. cv. S-1635). Int J Adv Res. 2013;1:22–6.
113. Li M, Shen L, Xu A, Miao X, Hou C, Sun P, Zhang Y, Huang Y. Genetic diversity among silkworm (*Bombyx mori* L., Lep., Bombycidae) germplasms revealed by microsatellites. Genome. 2005;48:802–10.
114. Li M, Hou C, Miao X, Xu A, Huang Y. Analyzing genetic relationships in *Bombyx mori* using intersimple sequence repeat amplification. Mol Entomol. 2007;100:202–2008.
115. Li Z, Yi Y, Yin X, Zhang Z, Liu J. Expression of foot-and mouth disease virus capsid proteins in silkworm-baculovirus expression system and its utilization as a subunit vaccine. PLoS ONE. 2008;3:e2273.
116. Li R, Liu L, Dominic K, Wang T, Fan T, Hu F, Wang Y, Zhang L, Li L, Zhao W. Mulberry (*Morus alba*) *MmSK* gene enhances tolerance to drought stress in transgenic mulberry. Plant Physiol Biochem. 2018;132:603–11.
117. Lin Z, Weiguo Z, Junbai C, Yong H, Xing JS, Liu L, Qiang S. Analysis of genetic diversity and construction of core collection of local mulberry varieties from Shanxi Province based on ISSR marker. Afr J Biotechnol. 2011;10:7756–65.
118. Liu C, Li J, Zhu P, Yu J, Hou J, Wang C, Long D, Yu M, Zhao A. Mulberry EIL3 confers salt and drought tolerances and modulates ethylene biosynthetic gene expression. Peer J. 2019;7:e6391.
119. Lizuka M, Ogawa S, Takeuchi A, Nakakita S, Kubo Y, Miyawaki Y, Hirabayashi J, Tomita M. Production of a recombinant mouse monoclonal antibody in transgenic silkworm cocoons. FEBS. 2009;276:5806–20.
120. Longvah T, Mangthya K, Ramulu P. Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricinii)* prepupae and pupae. Food Chem. 2011;128:400–3.
121. Longvah T, Manghtya K, Qadri SSYH. Eri silkworm: a source of edible oil with a high content of α-linolenic acid and of significant nutritional value. J Sci Food Agric. 2012;92:1988–93.
122. Lou CF, Zhang YZ, Zhou JM. Polymorphisms of genomic DNA in parents and their resulting hybrids in mulberry (*Morus*). Sericologia. 1998;38:437–45.
123. Lu M-C. Micropropagation of *Morus latifolia* poilet using axillary buds from mature trees. Sci Hortic. 2002;96:329–41.
124. Luckow VA, Lee SC, Barry GF, Olins PO. Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in *Escherichia coli*. J Virol. 1993;67:4566–79.
125. Lusser M, Parisi C, Plan D, Rodríguez-Cerezo E. Development of new technologies in plant breeding. Nat Biotechnol. 2012;30:231–9.
126. Ma X, Cao C, Li J, Zhu H. Novel prosthesis using silk fibroin for small calibre vascular. Key Eng Mater. 2005;288–289:461–4.
127. Ma X, Cao C, Zhu H. The biocompatibility of silk fibroin films containing sulfonated silk fibroin. J Biomed Mater. 2006;78:89–96.
128. Maeda S, Kawai T, Obinata M, Fujiwara H, Horiuchi T, Saeki Y, Sato Y, Furusawa M. Production of human α-interferon in silkworm using a baculovirus vector. Nature. 1985;315:592–4.
129. Mahendran M, Acharya C, Dash R, Ghosh AK, Kundu SC. Repetitive DNA in tropical tasar silkworm *Antheraea mylitta*. Gene. 2006;370:51–7.
130. Mandal BB, Kundu SC. Non-mulberry silk gland fibroin protein 3D scaffold for enhanced differentiation of human mesenchymal stem cells into osteocytes. Acta Biomater. 2009;5:2579–90.
131. Martinex-Zubiaur Y, Abreu MP, Hernández MCP, Sihler W, Falcao R, Ribeiro BM, de Souza ML. First record of a *Bombyx mori* nucleopolyhedrovirus (Bmnpv) isolate from cuba. J Curr Res. 2016;8:35766–70.
132. Masig CW, Ndabagye J, Barugahara I, Jaggwe R, Sempiri G, Nagamb MN, Lukoye D, Walimbwa E, Mushikoma D, Nabutsale A, Mutenyo M, Ekoot B, Nabende P, Amanya L, Mugabi R, Paul S, Kaslime G, Sabunyo N, Aine RS, Mugisha RR, Mousavi SMA, Ngoka B, Mugisha D, Nemeye P, Ndemere P. Country report, The 6th Asia-Pacific congress of sericulture and insect biotechnology. Mysore, India, 2–4th March 2019.
133. Masthan K, Rajkumar T, Narasimha Murthy CV. Studies on fortification of mulberry leaves with probiotics for improvement of silk quality. Int J Biotechnol Biochem. 2017;13:73–80.
134. Miao XX, Xub SJ, Li MH, Li MW, Huang JH, Dai FY, Marino SW, Mills DR, Zeng P, Mita K, Jia SH, Zhang Y, Liu WB, Xiang H, Guo QH, Xu AY, Kong XY, Lin HX, Shi YZ, Lu G, Zhang X, Huang W, Yasukochi Y, Sugasaki T, Shimada T, Nagaraju J, Xiang ZH, Wang SY, Goldsmith MR, Lu C, Zhao GP, Huang YP. Simple sequence repeat-based consensus linkage map of *Bombyx mori*. Proc Natl Acad Sci USA. 2005;102:16303–8.
135. Minagawa S, Nakaso Y, Tomita M, Igarashi T, Miura Y, Yasuda H, Sekiguchi S. Novel recombinant feline interferon carrying N-glycans with reduced allergy risk produced by a transgenic silkworm system. BMC Vet Res. 2018;14:260.
136. Ministry of Health, Labour and Welfare, Japan. Dietary reference intakes for Japanese. Daiichi-shup-pan. Tokyo (in Japanese); 2010.
137. Mirhoseini SZ, Rabiei B, Potki P, Dalirsefat SB. Linkage map construction for silkworm (*Bombyx mori* L.) based on amplified fragment length polymorphism markers. Iran J Biotechnol. 2009;7:28–36.
138. Mirhoseini SZ, Rabiei B, Potki P, Dalirsefat SB. Amplified fragment length polymorphism mapping of quantitative trait loci for economically important traits in the silkworm, *Bombyx mori*. J Insect Sci. 2010;10:Article 153.
139. Mishra S. Genetic analysis of traits controlling water use efficiency and rooting in mulberry (*Morus* spp.) by molecular markers. Ph.D. Thesis, University of Mysore, Mysuru, India. 2014. http://hdl.handle.net/10603/15951.
140. Mishra N, Hazarika NC, Narain K, Mahanta J. Nutritive value of non mulberry and mulberry silkworm pupae and consumption pattern in Assam. India J Nutr Res. 2003;23:1303–11.
141. Mishra S, Naik VG, Sukumar M, Pinto MV, Sheshashayee MS, Dandin SB. Genetic analysis of parental genotypes for mapping

of water use efficiency and root traits in mulberry. Indian J Genet Plant Breed. 2013;73:405–10.
142. Mita K, Morimyo M, Okano K, Koike Y, Nohata J, Kawasaki H, Kadono-Okuda K, Yamamoto K, Suzuki MG, Shimada T, Goldsmith MR, Maeda S. The construction of an EST database for *Bombyx mori* and its application. Proc Natl Acad Sci USA. 2003;100:14121–6.
143. Mita K, Kasahara M, Sasaki S, Nagayasu Y, Yamada T. The genome sequence of silkworm, *Bombyx mori*. DNA Res. 2004;11:27–35.
144. Miyashita V. A report on mulberry cultivation and training methods suitable to bivoltine rearing in Karnataka. Central Silk Board, Bangalore, India, 1986.
145. Mondal M, Tandon B, Radhakrishna PM. SeriNutrid-a balanced nutrient diet for silkworm (*Bombyx mori* L.) chawki rearing. Int J Adv Res Ideas Innov Technol. 2018;4:42–7.
146. Moorthy SM, Chandrakanth N, Ashwath SK, Kumar V, Bindroo BB. Genetic diversity analysis using RAPD marker in some silkworm breeds of *Bombyx mori* L. Ann Biol Res. 2013;4:82–8.
147. Motohashi T, Shimojima T, Fukagawa T, Maenaka K, Park EY. Efficient large-scale protein production of larvae and pupae of silkworm by *Bombyx mori* nuclear polyhedrosis virus bacmid system. Biochem Biophys Res Commun. 2005;326:564–9.
148. Muhonja L, Yamanouchi H, Yang CC, Kuwazaki S, Yokoi K, Kameda T, Sezutsu H, Jouraku A. Genome- wide SNP marker discovery and phylogenetic analysis of mulberry varieties using double digest restriction site associated DNA sequencing. Gene. 2020;726:144–62.
149. Muneta Y, Nagaya H, Minagawa Y, Enomoto C, Matsumoto S, Mori Y. Expression and one-step purification of bovine interleukin-21 (IL-21) in silkworm using a hybrid baculovirus expression system. Biotechnol Lett. 2004;26:1453–8.
150. Musataka SG. Increased applicability of genetically modified silkworms leads to innovations in sericulture and sericology. Country report. In: The 6th Asia-Pacific congress of sericulture and insect biotechnology, Mysore, India, 2–4th March 2019.
151. Nagaraja GM, Mahesh G, Satish V, Madhu M, Muthulakshmi M, Nagaraju J. Genetic mapping of Z chromosome and identification of W chromosome-specific markers in the silkworm, *Bombyx mori*. Heredity. 2005;95:148–57.
152. Nagaraju J. Recent advances in molecular genetics of the silk moth, *Bombyx mori*. Curr Sci. 1995;78:151–61.
153. Nagaraju J, Damodar KR, Nagaraja GM, Sethuraman BN. Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm, *Bombyx mori* L. Heredity. 2001;36:588–97.
154. Nagaya H, Kanaya T, Kaki H, Tobita Y, Takahashi M, Takahashi H, Yokomizo Y, Inumaru S. Establishment of a large-scale purification procedure for purified recombinant bovine interferon-τ produced by a silkworm-baculovirus gene expression system. J Vet Med Sci. 2004;66:1395–401.
155. Naik VG, Dandin SB. Identification of duplicate collections in the mulberry (*Morus* spp.) germplasm using RAPD analysis. Indian J Genet. 2006;66:287–92.
156. Naik VG, Sarkar A, Sathyanarayana N. DNA finger printing of Mysore local and V-1 cultivars of mulberry (*Morus* spp.) with RAPD markers. Indian J Genet. 2002;62:193–6.
157. Naik VG, Subbulakshmi N, Pinto MV, Mishra S, Qadri SMH. Assessment of genetic diversity among mulberry collections from South India using phenotypic and RAPD markers. Indian J Seric. 2013;52:34–43.
158. Naik VG, Thumilan B, Sarkar A, Dandin SB, Pinto MV, Sivaprasad V. Development of genetic linkage map of mulberry using molecular markers and identification of QTLs linked to yield and yield contributing traits. Sericologia. 2014;54:221–9.
159. Naik VG, Dandin SB, Tikader A, Pinto MV. Molecular diversity of wild mulberry (*Morus* spp.) of Indian subcontinent. Indian J Biotechnol. 2015;14:334–43.
160. Nair JS, Kumar SN, Nair KS. Improvement and Stabilization of feeding response to artificial diet in bivoltine pure stains of silkworm, *Bombyx mori* L. through directional selection. J Seric Technol. 2010;1:41–6.
161. Nataraju B, Sivaprasad V, Datta RK, Gupta SK, Shamim M. Colloidal textile dye-based dipstick immunoassay for the detection of nuclear polyhedrosis virus (BmNPV) of silkworm, *Bombyx mori* L. J Invertebr Pathol. 1994;63:135–9.
162. Nguu EK, Kadono-Okuda K, Mase K, Kosegawa E, Hara W. Molecular linkage map for the silkworm, *Bombyx mori*, based on restriction fragment length polymorphism of cDNA clones. J Insect Biotechnol Sericol. 2005;74:5–13.
163. Oka S, Tewary PK. Induction of hairy roots from hypocotyls of mulberry (*Morus indica* L.) by Japanese wild strains of *Agrobacterium rhizogenes*. J Seric Sci Jpn. 2000;69:13–9.
164. Opabode JT, Adebooye OC. Application of biotechnology for the improvement of Nigerian indigenous leaf vegetables. Afr J Biotechnol. 2005;4:138–42.
165. Orhan E, Ercisli S, Yildirim N, Agar G. Genetic variations among mulberry genotypes (*Morus alba*) as revealed by random amplified polymorphic DNA (RAPD) markers. Plant Syst Evol. 2007;265:251–8.
166. Orhan E, Akin M, Eyduran SP, Ercisli S. Molecular characterization of mulberry genotypes and species in Turkey. Not Bot Hortic Agrobot Cluj Napoca. 2020;48:557–79.
167. Ozrenk K, Sensoy RIG, Erdinc C, Guleryuz M, Aykanat A. Molecular characterization of mulberry germplasm from Eastern Anatolia. Afr J Biotechnol. 2010;9:1–6.
168. Padamwar MN, Pawar AP, Daithankar AV, Mahadik KR. Silk sericin as amoisturizer: an in vivo study. J Cosmet Dermatol. 2005;4:250–7.
169. Patra C, Talukdar S, Novoyatleva T, Velagala SR, Mühlfeld C, Kundu B, Kundu SC, Engel FB. Silk protein fibroin from *Antheraea mylitta* for cardiac tissue engineering. Biomaterials. 2012;33:2673–80.
170. Pinto MV, Naik VG, Qadri SMH. Genetic variability studies in mulberry using microsatellite markers. J Sericult Technol. 2012;3:38–43.
171. Prasad MD, Muthulakshmi M, Madhu M, Archak S, Mita K, Nagaraju J. Survey and analysis of microsatellites in the silkworm, *Bombyx mori*: frequency, distribution, mutations, marker potential and their conservation in heterologous species. Genetics. 2005;169:197–214.
172. Promboon A, Shimada T, Fujiwara F, Kobayashi M. Linkage map of random amplified polymorphic DNAs (RAPDs) in the silkworm *Bombyx mori*. Genet Res. 1995;66:1–7.
173. Qian Q, You Z, Ye L, Che J, Wang Y, Wang S, Zhong B. High-efficiency production of human serum albumin in the posterior silk glands of transgenic silkworms, *Bombyx mori* L. PLoS ONE. 2018;13:e0191507.
174. Qiana H, Lia G, Zhaoa G, Liua M, Suna P, Xua A. Fingerprint analysis of *Bombyx mori* local variety resources based on SSR markers. Sci Asia. 2019;45:342–9.
175. Qiuxia D, Yang L, Kotoka DK, Weiguo Z. Molecular cloning and abiotic stress expression analysis of gtpase era gene in mulberry (*Morus Alba* L.). J Agric Res. 2020;5:000239.
176. Radjabi R, Sarafrazi A, Tarang A, Kamali K, Tirgari S. Intraspecific biodiversity of Iranian local races of silkworm *Bombyx mori* by ISSR (inter-simple sequence repeat) molecular marker. World J Zool. 2012;7:17–22.
177. Raghunath MK, Lal S, Khurana P. In vitro plant regeneration from different explants of elite mulberry (*Morus* sp.) genotypes. Bangladesh J Seric. 2009;2:31–9.

178. Raghunath MK, Nataraj KN, Meghana JS, Sanjeevan RS, Rajan MV, Qadri SMH. In vitro plant regeneration of *Morus indic*a L. cv. V-1 using leaf explants. Am J Plant Sci. 2013;4:2001–5.
179. Ramesha C, Kumari SS, Anuradha CM, Lakshmi H, Kumar CS. Nutrigenomic analysis of mulberry silkworm (*Bombyx mori* L.) strains using polymerase chain reaction-simple sequence repeats (PCR-SSR). Int J Biotechnol Mol Bio Res. 2010;1:92–100.
180. Rangacharyulu PV, Giri SS, Paul BN, Yashoda KP, Rao RJ, Mahendrakar NS, Mohanty SN, Mukhopadhyay PK. Utilization of fermented silkworm pupae silage in feed for carps. Bioresour Technol. 2003;86:29–32.
181. Rao AP. Some salient features of Andhra local ecoraces of *Antheraea mylitta* Drury in relation to its conservation and multiplication. Int J Wild Silkmoth Silk. 2000;5:356–8.
182. Rao P, Nuthan D, Krishna KS. A protocol for in vitro regeneration of rainfed mulberry varieties through callus phase. Eur J Biol Sci. 2010;2:80–6.
183. Roh DH, Kang SY, Kim JY, Kwon YB, Hae YK, Lee KG, Park YH, Baek RM, Heo CY, Choe J, Lee JH. Wound healing effect of silk fibroin/alginate-blended sponge in full thickness skin defect of rat. J Mater Sci Mater Med. 2006;17:547–52.
184. Rohela GK, Shabnam AA, Shukla P, Kamili AN, Ghosh MK. Rapid one step protocol for the in vitro micropropagation of *Morus multicaulis* Var. Goshoerami, an elite mulberry variety of temperate region. J Exp Biol Agric Sci. 2018;6:936–46.
185. Rohela GK, Jogam P, Mir MY, Shabnam AA, Shukla P, Abbagani S, Kamili AN. Indirect regeneration and genetic fidelity analysis of acclimated plantlets through SCoT and ISSR markers in *Morus alba* L. cv. Chinese white. Biotechnol Rep. 2020;25:e00417.
186. Roy G, Mandal K, Ravikumar G. PCR-based detection of microsporidia in silkworms using non-conventional RNA polymerase primers. Biosci Biotechnol Res Commun. 2017;10:676–9.
187. Saeed B, Das M, Haq QMR, Khurana P. Overexpression of beta carotene hydroxylase-1 (bch1) in mulberry, *Morus indica* cv. K-2, confers tolerance against high-temperature and high irradiance stress induced damage. Plant Cell Tissue Organ Cult. 2015;120:1003–15.
188. Saeed B, Baranwal VK, Khurana P. Comparative transcriptomics and comprehensive marker resource development in mulberry. BMC Genom. 2016;17:1–14.
189. Saha S, Adhikari S, Dey T, Ghosh PD. RAPD and ISSR based evaluation of genetic stability of micropropagated plantlets of *Morus alba* L. variety S-1. Meta Gene. 2016;7:7–15.
190. Sahay A, Satpathy S, Sharan SK. Field trial experiment of artificial diet on tasar silkworm, *Anthereaea myltta* D. Nat Prec. 2011. https://doi.org/10.1038/npre.2011.6701.1.
191. Sajeevan RS, Jeba Singh S, Nataraja KN, Shivanna MB. An efficient in vitro protocol for multiple shoot induction in mulberry, *Morus alba* L. variety V1. Int Res J Plant Sci. 2011;2:254–61.
192. Sajeevan RS, Nataraja KN, Shivashankara KS, Pallavi N, Gurumurthy DS, Shivanna MB. Expression of *Arabidopsis* SHN1 in Indian mulberry (*Morus indica* L.) increases leaf surface wax content and reduces post-harvest water loss. Front Plant Sci. 2017;8:418.
193. Sanjeeva Reddy K, Mahalingam CM, Murugesh KA, Mohankumar S. Exploring the genetic variability in *Bombyx mori* L. with molecular marker. Karnataka J Agric Sci. 2009;22:479–83.
194. Saotome T, Hayashi H, Tanaka R, Kinugasa A, Uesugi S, Tatematsu K, Sezutsu H, Kuwabara N, Asakura T. Introduction of VEGF or RGD sequences improves revascularization properties of *Bombyx mori* silk fibroin produced by transgenic silkworm. J Mater Chem B. 2015;35:7109–16.
195. Sarkar A, Kumar JS, Datta RK. Gradual improvement of mulberry varieties under irrigated conditions in South India and the optimal program for varietal selection in the tropics. Sericologia. 2000;40:449–61.
196. Selvakumar T, Nataraju B, Balvenkatsubbaiah M, Sivaprasad V, Baig M, Kumar V, Sharma SD, Thiagrajan V, Datta RK. A report on the prevalence of silkworm diseases and estimated crop loss. In: Dandin SB, Gupta VP, editors. Strategies for sericulture research and development. CSRTI: Mysore; 2002. p. 354–7.
197. Shamim M, Baig M, Nataraju B, Datta RK, Gupta SK. Evaluation of protein-a linked monoclonal antibody latex agglutination test for diagnosis of nuclear polyhedrosis virus (BmNPV) of silkworm *Bombyx mori* L. J Immunoassay. 1995;1995(16):155–66.
198. Sharma A, Sharma R, Machii H. Assessment of genetic diversity in a *Morus* germplasm collection using fluorescence-based AFLP markers. Theor Appl Genet. 2000;101:1049–55.
199. Sharma S, Bano S, Ghosh AS, Mandal M, Kim HW, Dey T, Kundu SC. Silk fibroin nanoparticles support in vitro sustained antibiotic release and osteogenesis on titanium surface. Nanomed Nanotechnol Biol Med. 2016;12:1193–204.
200. Sheet S, Ghosh K, Acharya S, Kim K-P, Lee YS. Estimating genetic conformism of korean mulberry cultivars using random amplified polymorphic DNA and inter-simple sequence repeat profiling. Plants. 2018;7:21.
201. Shi J, Heckel DG, Goldsmith MR. A genetic linkage map for the domesticated silkworm, *Bombyx mori*, based on restriction fragment length polymorphisms. Genet Res Camb. 1995;66:109–26.
202. Shruti AJ, Hadimani DK, Sreenivas AG, Beladhadi RV. Effect of probiotic feed supplements to mulberry silkworm, *Bombyx mori* L. for larval growth and development parameters. Int J Chem Stud. 2019;7:3914–9.
203. Sinha RK, Ramesha MN, Gowda V, Vathsala TV, Perugu AY. Seir-states of India—a profile. New Delhi: Astral International Pvt. Ltd.; 2019.
204. Sreekumar S, Ashwath SK, Slathia M, Kumar SN, Qadri SMH. Detection of a single nucleotide polymorphism (SNP) DNA marker linked to cocoon traits in the mulberry silkworm, *Bombyx mori* (Lepidoptera: *Bombycidae*). Eur J Entomol. 2011;108:347–54.
205. Srivastava PP, Vijayan K, Awasthi AK, Saratchandra B. Genetic analysis of *Morus alba* through RAPD and ISSR markers. Indian J Biotechnol. 2004;3:527–32.
206. Srivastava PP, Vijayan K, Awasthi AK, Kar PK, Thangavelu K, Saratchandra B. Genetic analysis of silkworms (*Bombyx mori*) through RAPD markers. Indian J Biotechnol. 2005;4:389–95.
207. Srivastava PP, Vijayan K, Kar PK, Saratchandra B. Diversity and marker association in tropical silkworm breeds of *Bombyx mori* (Lepidoptera: *Bombycidae*). Int J Trop Insect Sci. 2011;31:182–91.
208. Staub JE, Serquen FC. Genetic markers, map construction, and their application in plant breeding. Hortic Sci. 1996;31:729–41.
209. Subbaiah EV, Royer C, Kanginakudru S, Satyavathi VV, Babu AB, Sivaprasad V, Chavancy G, DaRocha M, Jalabert A, Mauchamp B, Basha I, Couble P, Nagaraju J. Engineering silkworms for resistance to baculovirus through multigene RNA interference. Genetics. 2013;193:63–75.
210. Sugihara A, Sugiura K, Morita H, Ninagawa T, Tubouchi K, Tobe R, Izumiya M, Horio T, Abraham NG, Ikehara S. Promotive effects of a silk film on epidermal recovery from full-thickness skin wounds. Proc Soc Exp Biol Med. 2000;225:58–64.
211. Sugimura Y, Miyazaki J, Yonebayashi K, Kotani E, Furusawa T. Gene transfer by electroporation into protoplasts isolated from mulberry call. J Seric Sci Jpn. 1999;68:49–53.
212. Suraporn S, Sangsuk W, Chanhan P, Promma S. Effects of probiotic bacteria on the growth parameters of the Thai silkworm, Bombyx mori. Thai J Agric Sci. 2015;48:29–33.
213. Susheelamma BN, Jolly MS, Sengupta K, Venkateswarlu M, Suryanarayana N. Quantitative assessment of drought resistant

mulberry genotype leaves cultivated under natural stress (low rain fall) conditions by feeding larvae of *Bombyx mori* L. In: Sampath J, editor. Proceedings of the International congress on tropical sericulture practice. Central Silk Board, Bangalore; 1988. p. 89–101.
214. Tada MY, Tatematsu KY, Ishii-Watabe A, Harazono A, Takakura D, Hashii N, Sezutsu H, Kawasak N. Characterization of anti-CD20 monoclonal antibody produced by transgenic silkworms (*Bombyx mori*). MAbs. 2015;7:1138–50.
215. Taha H, Ghazy UM, Gabr AMM, El-Kazzaz AAA, Ahmed EAMM, Haggag KM. Optimization of in vitro culture conditions affecting propagation of mulberry plant. Bull Natl Res Cent. 2020. https://doi.org/10.1186/s42269-020-00314-y.
216. Talebi E, Khademi M, Subramanya G. RAPD markers for understanding of the genetic variability among the four silkworm races and their hybrids. Middle East J Sci Res. 2011;7:789–95.
217. Tamura T, Thibert C, Royer C, Kanda T, Abraham E, Kamba M, Komoto N, Thomas JL, Mauchamp B, Chavancy G, Shirk P, Fraser M, Prudhomme JC, Couble P. Germline transformation of the silkworm *Bombyx mori* L. using a PiggyBac transposon-derived vector. Nat Biotechnol. 2000;18:81–4.
218. Tan Y-D, Wan C, Zhu Y, Lu C, Xiang Z, Deng H-W. An amplified fragment length polymorphism map of the silkworm. Genetics. 2001;157:1277–84.
219. Tekin QK, Sezer D, Soganci K, Tunca RI. Molecular and microscopic detection of microsporidia in some silkworm (*Bombyx mori* L.) populations in Turkey. Turk J Agric Nat Sci. 2014;1:450–3.
220. Thomas TD. Thidiazuron induced multiple shoot induction and plant regeneration from cotyledonary explants of mulberry. Biol Plant. 2003;46:529–33.
221. Thomas TD, Bhatnagar AK, Bhojwani SS. Production of triploid plants of mulberry (*Morus alba* L.) by endosperm culture. Plant Cell Rep. 2000;19:395–9.
222. Thorpe TA. Biotechnological applications of tissue culture to forest tree improvement. Biotechnol Adv. 1983;1:263–78.
223. Thumilan BM, Kadam NN, Biradar J, Sowmya HR, Mahadeva A, Madhura JN, Makarla U, Khurana P, Sreeman SM. Development and characterization of microsatellite markers for *Morus* spp. and assessment of their transferability to other closely related species. BMC Plant Biol. 2013;13:194. https://doi.org/10.1186/1471-2229-13-194.
224. Thumilan BM, Sajeevan RS, Biradar J, Madhuri T, Nataraja KN, Sreeman SM. Development and characterization of genic SSR markers from Indian mulberry transcriptome and their transferability to related species of *Moraceae*. PLoS ONE. 2016;11:e0162909. https://doi.org/10.1371/journal.pone.0162909.
225. Tikader A, Dandin SB. DNA fingerprint of inter and intraspecific hybrids from *Morus* species using RAPD. Geobios. 2008;35:113–20.
226. Tikader A, Kamble CK. Mulberry wild species in India and their use in crop improvement—a review. Aust J Crop Sci. 2008;2:64–72.
227. Tomita M, Munetsuna H, Sato T, Adachi T, Hino R, Hayashi M, Shimizu K, Nakamura N, Tamura T, Yoshizato K. Transgenic silkworms produce recombinant human type III procollagen in cocoons. Nat Biotechnol. 2003;21:52–6.
228. Trivedy K, Nair KS, Ramesh M, Nisha G, Nirmal SK. New semi-synthetic diet "Nutrid"-a technology for rearing young instar silkworm in India. Indian J Seric. 2003;42:158–61.
229. Ukaji N, Kuwabara C, Takezawa D, Arakawa K, Yoshida S, Fujikawa S. Accumulation of small heat shock protein in the endoplasmic reticulum of cortical parenchyma cells in mulberry in association with seasonal cold acclimation. Plant Physiol. 1999;120:481–9.
230. Ukaji N, Kuwabara C, Takezawa D, Arakawa K, Fu-jikawa S. Cold acclimation-induced WAP27 localized in endoplasmic reticulum in cortical parenchyma cells of mulberry tree was homologous to group 3 late embryogenesis abundant proteins. Plant Physiol. 2001;126:1588–97.
231. Umate P, Rao KV, Kiranmayee K, Jaya Sree T, Sadanandam A. Plant regeneration of mulberry (*Morus indica*) from mesophyll derived protoplasts. Plant Cell Tissue Organ Cult. 2005;82:289–93.
232. Vanapruk P, Attathom T, Sanbatsiri K, Attathom S. Comparison of methods for the detection of nuclear polyhedrosis virus in silkworm, *Bombyx mori* Linn. In: Proceedings of the 30th Kasetsart University annual conference: plant science, Kasetsart University, Bangkok, Thailand; 1992. pp. 237–243.
233. Varada B, Pradeep ANR, Awasthi AK, Sivaprasad V, Ponnuvel KM, Mishra RK. Non-target host immune gene modulation in transgenic silkworm *Bombyx mori* endowed with RNAi silence *Bm*NPV genes. Biotechnol J Int. 2017;20:1–12.
234. Vasil IK. A history of plant biotechnology: from the cell theory of Schleiden and Schwann to biotech crops. Plant Cell Rep. 2008;2008(27):1423–40.
235. Velu D, Ponnuvel KM, Muthulakshmi M, Sinha RK, Qadri SMH. Analysis of genetic relationship in mutant silkworm strains of *Bombyx mori* using inter simple sequence repeat (ISSR) markers. J Genet Genom. 2008;35:291–7.
236. Venkateswarlu M, Saratchandra B, Surendranath B, Vijayan K, Rajeurs S. Strategies for genome mapping and marker assisted selection in mulberry. J Cytol Genet. 2005;5:111–24.
237. Venkateswarlu M, Raje US, Surendra NB, Shashidhar HE, Maheswaran M, Veeraiah TM, Sabitha MG. A first genetic linkage map of mulberry (*Morus* spp.) using RAPD, ISSR, and SSR markers and pseudotestcross mapping strategy. Tree Genet Genomes. 2006;3:15–24.
238. Vijayan K. Genetic relationships of Japanese and Indian mulberry (*Morus* spp.) revealed by DNA fingerprinting. Plant Syst Evol. 2004;243:221–32.
239. Vijayan K. Molecular markers and their application in mulberry breeding. Int J Ind Entomol. 2007;15:01–11.
240. Vijayan K, Chatterjee SN. ISSR profiling of Indian cultivars of mulberry (*Morus* spp.) and its relevance to breeding programs. Euphytica. 2003;131:53–63.
241. Vijayan K, Chakraborti SP, Roy BN. Plant regeneration from leaf explants of mulberry: influence of sugar, genotype and 6-benzyladenine. Indian J Exp Biol. 2000;38:504–8.
242. Vijayan K, Srivastava PP, Awasthi AK. Analysis of phylogenetic relationship among five mulberry (*Morus*) species using molecular markers. Genome. 2004;47:439–48.
243. Vijayan K, Kar PK, Tikader A, Srivastava PP, Awasthi AK, Thangavelu K, Saratchandra B. Molecular evaluation of genetic variability in wild populations of mulberry (*Morus serrata* Roxb.). Plant Breed. 2004;123:568–72.
244. Vijayan K, Awasthi AK, Srivastava PP, Saratchandra B. Genetic analysis of Indian mulberry varieties through molecular markers. Hereditas. 2004;141:8–14.
245. Vijayan K, Chatterjee SN, Nair CV. Molecular characterization of mulberry genetic resources indigenous to India. Genet Resour Crop Evol. 2005;52:77–86.
246. Vijayan K, Nair CV, Kar PK, Madhusudan TP, Saratchandra B, Raje US. Genetic variability within and among three ecoraces of the tasar silkworm *Antheraea mylitta* drury as revealed by ISSR and RAPD markers. Int J Ind Entomol. 2005;10:283–90.
247. Vijayan K, Srivastava PP, Nair CV, Tikader A, Awasthi AK, Urs SR. Molecular characterization and identification of markers associated with leaf yield traits in mulberry using ISSR markers. Plant Breed. 2006;125:298–301.

248. Vijayan K, Nair CV, Chatterjee SN. Diversification of mulberry (*Morus indica* var. S36), a vegetatively propagated tree species. Casp J Environ Sci. 2009;7:23–30.
249. Vijayan K, Nair CV, Urs SR. Assessment of genetic diversity in the tropical mulberry silkworm (*Bombyx mori* L.) with mtDNA-SSCP and SSR markers. Emir J Food Agric. 2010;22:71–83.
250. Vijayan KA, Tikader A, da Silva JAT. Application of tissue culture techniques for propagation and crop improvement in mulberry (*Morus* spp.). Tree For Sci Biotechnol. 2011;5:1–13.
251. Vijayan K, Raju PJ, Tikader A, Saratchnadra B. Biotechnology of mulberry (*Morus* L.)—a review. Emir J Food Agric. 2014;26:472–96.
252. Vijaykumar A, Patil GM, Kavita MB. Effective concentration of azolla supplementation to grown up silkworms. Adv Life Sci. 2016;5:4029–38.
253. Vlaic B, Marghitas LA, Vlaic A, Raica P. Analysis of genetic diversity of mulberry silkworm (*Bombyx mori* L.) using RAPD molecular markers. Anim Sci Biotechnol. 2012;69:292–6.
254. Vootla SK, Lu MX, Kari N, Gadwala M, Lu Q. Rapid detection of infectious flacherie virus of the silkworm, *Bombyx mori*, using RT-PCR and nested PCR. J Insect Sci. 2013;13:120.
255. Wang H, Lou C, Zhang Y, Tan J, Jiao F. Preliminary report on *Oryza cystatin* gene transferring into mulberry and production of transgenic plants. Acta Sericologica Sin. 2003;29:291–4.
256. Wang Z, Zhang Y, Dai F, Luo G, Xiao G, Tang C. Genetic diversity among mulberry genotypes from seven countries. Physiol Mol Biol Plants. 2017;23:421–7.
257. Wangari NP, Gacheri KM, Theophilus MM, Lucas N. Use of SSR markers for genetic diversity studies in mulberry accessions grown in Kenya. Int J Biotechnol Mol Biol Res. 2013;4:38–44.
258. Wani SA, Bhat MA, Malik GN, Zaki FA, Mir MR, Wani N, Bhat KM. Genetic diversity and relationship assessment among mulberry (*Morus* spp.) genotypes by simple sequence repeat (SSR) marker profile. Afr J Biotechnol. 2013;12:3181–7.
259. Wei GQ, Yu L, Liu CL, Zhu BJ, Ding HJ. Linkage and mapping analyses of the normal marking gene *+P* in the silkworm (*Bombyx mori*) using SSR markers. Genet Mol Res. 2013;12:2351–9.
260. Wei-Guo Z, Yile P, Shihai ZZJ, Xuexia M, Yongping H. Phylogeny of the genus *Morus* (Urticales: Moraceae) inferred from ITS and *trn*L-F sequences. Afr J Biotechnol. 2005;4:563–9.
261. Wei-Guo Z, Xue-Xia M, Bo Z, Lin Z, Yi-Le P, Yong-Ping H. Construction of fingerprinting and genetic diversity of mulberry cultivars in China by ISSR markers. Acta Genetica Sin. 2006;33:851–60.
262. Weiguo Z, Yile P. Genetic diversity of genus *Morus* revealed by RAPD markers. Int J Agric Biol. 2004;6:950–5.
263. Weiguo Z, Zhihua Z, Xuexia M, Yong Z, Sibao W, Jianhua H, Hui X, Yile P, Yongping H. A comparison of genetic variation among wild and cultivated *Morus* species (Moraceae: *Morus*) as revealed by ISSR and SSR markers. Biodivers Conserv. 2007;16:275–90.
264. Wulandari YRE, Harjosudirjo MA. Micropropagation of *Morus cathayana* through in vitro culture from local Bogor, West Java, Indonesia. Nu Biosci. 2019;11:18–22.
265. Xu Y, Wang H, Qin R, Fang L, Liu Z, Yuan S, Gai Y, Ji X. Characterization of NPR1 and NPR4 genes from mulberry (*Morus multicaulis*) and their roles in development and stress resistance. Physiol Plant. 2019;167:302–16.
266. Xuan Y, Wu Y, Li P, Liu R, Luo Y, Yuan J, Xiang Z, He N. Molecular phylogeny of mulberries reconstructed from ITS and two cpDNA sequences. PeerJ. 2019;7:e8158.
267. Yamamoto K, Narukawa J, Kadono-Okuda K, Nohata J, Sasanuma M, Suetsugu Y, Banno Y, Fujii H, Goldsmith MR, Mita K. Construction of a single nucleotide polymorphism linkage map for the silkworm, *Bombyx mori*, based on bacterial artificial chromosome end sequences. Genetics. 2006;173:151–61.
268. Yamamoto K, Nohata J, Kadono-Okuda K, Narukawa J, Sasanuma M, Sasanuma SI, Minami H, Shimomura M, Suetsugu Y, Banno Y, Osoegawa K, de Jong PJ, Goldsmith MR, Mita K. A BAC-based integrated linkage map of the silkworm *Bombyx mori*. Gen Biol. 2008;9:R21. https://doi.org/10.1186/gb-2008-9-1-r21.
269. Yang HX, Zhu XR, Lu HS. Research progress on application of silkworm pupas in medical science. Bull Sci Technol. 2002;18:318–22.
270. Yasukochi Y. A dense genetic map of the silkworm, *Bombyx mori*, covering all chromosomes based on 1018 molecular markers. Genetics. 1998;150:1513–25.
271. Yasukochi Y. A simple and accurate method for generating codominant markers: an application of conformation-sensitive gel electrophoresis to linkage analysis in the silkworm. Mol Gen Genet. 1999;261:796–802.
272. Zaki M, Kaloo ZA, Sofi M. Micropropagation of *Morus nigra* L. from nodal segments with axillary buds. World J Agri Sci. 2011;7:496–503.
273. Zhan S, Huang J, Guo Q, Zhao Y, Li W, Miao X, Goldsmith MR, Li M, Huang Y. An integrated genetic linkage map for silkworms with three parental combinations and its application to the mapping of single genes and QTL. BMC Genom. 2009;10:389.
274. Zhao W, Pan Y. Genetic diversity of genus *Morus* revealed by RAPD markers in China. Int J Agric Biol. 2004;6:950–4.
275. Zhao W, Miao X, Jia S, Pan Y, Huang Y. Isolation and characterization of microsatellite loci from the mulberry, *Morus* L. Plant Sci. 2005;16:519–25.
276. Zhao W, Zhou Z, Miao X, Wang S, Zhang L, Pan Y, Huang Y. Genetic relatedness among cultivated and wild mulberry (Moraceae: *Morus*) as revealed by inter-simple sequence repeat (ISSR) analysis in China. Can J Plant Sci. 2006;86:251–7.
277. Zhao W, Wang Y, Chen T, Jia G, Wang X, Qi J, Pang Y, Wang S, Li Z, Huang Y, Pan Y, Yang Y-H. Genetic structure of mulberry from different ecotypes revealed by ISSRs in China: an implications for conservation of local mulberry varieties. Sci Hortic. 2007;115:47–55.
278. Zhao W, Fang R, Pan Y, Yang Y, Chung JW, Chung IM, Park YJ. Analysis of genetic relationships of mulberry (*Morus* L.) germplasm using sequence-related amplified polymorphism (SRAP) markers. Afr J Biotechnol. 2009;8:2604–10.
279. Zhao A, Zhao T, Zhang Y, Xia Q, Lu C, Zhou Z, Xiang Z, Nakagaki M. New and highly efficient expression systems for expressing selectively foreign protein in the silk glands of transgenic silkworm. Transgenic Res. 2010;19:29–44.
280. Zhao X, Wei G, Liu C, Zou C, Zhu B. Linkage and mapping analyses of the no glue egg gene Ng in the silkworm (*Bombyx mori* L.) using simple sequence repeats (SSR) markers. Afr J Biotechnol. 2011;10:9549–56.