A Textbook on Silkworm Rearing Technology

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CHAPTER 1
FUNCTIONING OF CENTRAL SILK BOARD & NOTE ON SERICULTURE

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ABSTRACT:
The Central Silk Board has established 8 Regional Offices in New Delhi, Mumbai, Kolkata, Hyderabad, Bhubaneshwar, Guwahati, Lucknow, and Patna in order to coordinate the sericulture development initiatives in various States and to carry out pre-shipment inspection of silk items intended for export. To coordinate the transmission of technology, Regional Offices of CSB work closely with State Sericulture Departments, field units, and CSB field functionaries. Regional Offices also organise meetings of the Central Silk Board's State Level Sericulture Coordination Committee. As of January 1st, 2019, the CSB has 2,781 employees on staff. The CSB's mandated activities include research and development, upkeep of a four-tier network for the production of silkworm seeds, leadership in commercial production of silkworm seeds, standardisation and implementation of quality standards in various production processes, and advice to the government on all issues relating to sericulture and the silk industry. Through an integrated Central Sector plan called "Silk Samagra," an integrated plan for the development of the silk industry, the 192 units of the Central Silk

KEYWORDS:
Field, Mulberry, Research, Silkworm, Tasar.

INTRODUCTION

Act No. LXI of 1948, an Act of Parliament, created the Central Silk Board (CSB), a Statutory Body, in 1948. It operates under the administrative supervision of the Government of India's Ministry of Textiles, which is headquartered in Bangalore. The Board is made up of 39 individuals who were chosen for three years in accordance with the authority granted by Sub-Section 3 of Section 4 of the CSB Act 1948. The Central Government will appoint the Board's Vice-Chairperson and Secretary, both of whom must be officers with at least the rank of Joint Secretary to the Government. The Central Government will also nominate the Chairperson of the Board and two other officials, one of whom will be the head of the Silk Division in the Ministry of Textiles[1], [2].

Board are carrying out these specified operations of the CSB. Silk Samagra has the following four components:

1. Initiatives in research and development, training, technology transfer, and information technology.

2. The Seed Organisation.

3. Market expansion and coordination.

4. Systems for quality certification, export, brand promotion, and technology advancement.

Initiatives in research & development, training, transfer of technology, and I.T.

Through creative methods, the Research & Training Institutes of the CSB encourage scientific and technical advancements in output and productivity for sustainable sericulture.

Mulberry sericulture is the primary focus of the major institutions in Mysore (Karnataka), Berhampore (West Bengal), and Pampore (J&K), while Tasar culture is the focus of Ranchi (Jharkhand), Muga and Eri culture is the focus of Lahdoigarh, Jorhat (Assam), and Ranchi (Jharkhand) institutes. Regional Sericulture Research Stations have been operating to build a technological package appropriate to the area and disseminate research results in accordance with regional demands. Additionally, a network of Research Extension Centres (RECs) and its subunits seek to help sericulturists in their extension activity[3], [4].

The Board has created a Central Silk Technological Research Institute in Bangalore to boost R&D in the post-cocoon industry. The CSB has also established the Seri-Biotech Research Laboratory in Bangalore (Karnataka), the Central Sericultural Germplasm Resource Centre in Hosur (Tamil Nadu), and the Silkworm Seed Technology Laboratory in Bangalore.The various R&D institutes of CSB have started a total of 16 new research projects during the 2018–19 academic year, and 30 projects have been completed as of the end of December 2018. Currently, a total of 98 research projects, including 58 in the Mulberry Sector, 30 in the Vanya Sector, and 10 in the Post Cocoon Sector, are in various stages of completion[5], [6].

Highlights of Research Programmes in Research & Development

R&D on the host plant, the mulberry:

The commercial exploitation of the varieties G4 for the South, C2038 for the East and North regions, and Tr-23 for the mountainous parts of the East and North-Eastern zone. Found three genotypes of mulberry with high leaf production and tolerance to low temperature stress: C-108 (15.4 mt), C-384 (9.7 mt), and C-212 (9.2 mt). With leaf yields of 49.9 & 34.0 t/ha/yr under full & half of NPK dosage above 44.1 & 26.8 t/ha/yr of S-1635 variety in West Bengal Condition, the developed mulberry hybrid C-9 is appropriate for low input conditions.

In West Bengal, Assam, and other Eastern and North Eastern States, the mulberry cultivar C-2028, which can withstand standing water, is becoming more and more well-known. Under the circumstances of West Bengal, five genotypes—C-01, C-384, C-11, C-02, and C-05—were shown to have superior growth characteristics and leaf yield to the control variety S-1635. Identified three putative cold tolerant genes for the development of cold tolerant mulberry varieties for temperate regions. Three new mulberry varieties, AGB-8, PPR-1, and C1360 (Ganga), have been shortlisted for testing in the next phase of All India Coordinated Experimental Trials for Mulberry (AICEM).

The leaf roller (Diaphania pulverulentalis) male insects may be caught using the pheromone chemicals Z-11 hexadecenal, Octadecane, and Z-e-7, 11- hexadecenal acetate. EAG is currently being developed, along with the pheromone molecule. In order to amplify the genes for Nitrogen Reductase and Chalconne Synthase, DNA from 110 progeny was taken. Induction of callus from the fusion of four different mulberry variety protoplasts. Vishala, a legal kind of mulberry, is now gaining popularity in Eastern and North-Eastern regions. In West Bengal, under irrigated circumstances, Vishala recorded a 12.21% greater leaf yield, or 10267 kg/ha/crop, above S1635, or 9150 kg/ha/crop.

DISCUSSION

There were issued 17236 soil health cards. By adding 55 additional collections, the number of mulberry accession being conserved at CSGRC Hosur has climbed to 1291. With an effectiveness of 88-94% disease suppression, a novel formulation called "Rot fix" was created to treat root rot. Ankur, a mix of organic and inorganic nutrients, was advised since it

increased the output of cocoons and mulberry leaves by 11% and 14%, respectively. A mulberry pest incidence calendar for various agro-climates in the Eastern and North Eastern areas was established in order to control mulberry pests effectively.In the field, the bio-nematicide "Nemahari" reduced root knot disease by up to 80% while improving leaf production by 15% to 18%. Investigated the prevalence of root rot infections in Kashmir areas and determined that Helicobasidium mompa and Fusarium oxiforum were the responsible organisms. The field performance of mulberries at various spacings revealed that the best production occurred at (150+90) cm x 60 cm (13174.83 kg/ha/crop), while the lowest yield occurred at 270 cm × 60 cm (8842.17 kg/ha/crop). Mulberry productivity increased from 50 MT/Ha/yr in 2005–2006 to 60 MT/Ha/yr in 2017–18 because to R&D initiatives[7], [8].

Research and Development on Mulberry Silkworm: The Hybrid Authorization Committee proposed the approval of two novel bivoltine hybrids, G11xG19 and B.con1 x B.con4, with cocoon yields of 68.5kg/100 dfls and 58kg/100 dfls, respectively. The procedure of notifying the Gazette is now underway.A large-scale, multi-location study including MV1xS8 and S8x CSR16 is now being conducted at the farmer level. For a small number of field tests, N21 x N56, a thermo-tolerant bivoltine double hybrid with a yield potential of 72 kg/100 dfls & 21.5% shell, was chosen. Using SSR markers (LFL0329 & LFL1133) related to thermo-tolerance, four thermo-tolerant silkworm lines were created. Two bivoltine hybrids, CSR52N x CSR26N and (CSR52NxS8N) x (CSR16NxCSR26N), that are NPV-tolerant have been shortlisted for field testing. To test the potential for yield and thermotolerance, two enhanced crossbreeds—L3 x S8 and HB4 x S8—that are resistant to high temperatures and BmNPV, have >90% pupation rates, 20–21% shell ratios, and 14–15% raw silk—were chosen for field trials. Four hybrids were chosen for fall raising in North India: CSR46 APS9; APS9 BBE 198; APS5 APS9; and SK6 SK7[9], [10].

Through shuttle breeding, two novel bivoltine single hybrids, BHP-2 x BHP-8 and BHP-3 x BHP-8, were created with greater shell percentages than the current hybrids. For the Eastern area, novel bivoltine silkworm hybrids Gen-3 x SK6 and M6DPC x (SK6 x SK7) with cocoon production potential of 50–55 kg yield/100 dfls and 45–50 kg yield/100 dfls, respectively, were produced. RNAi from the transgenic silkworm Nistari was introduced into CSR4 and CSR27 to create NPV resistant strains. These CSR4 and CSR27 lines demonstrated greater NPV resistance. Three silkworm breeds, including APS-5, APS-HTP5, and BBE198, were discovered as DNV resistant based on the presence or lack of the DNV resistant gene nsd-2.

Pichia pastoris was used to create and clone a full-length lipoprotein gene for the extraction and characterisation of recombinant proteins. To track the prevalence of illness in South Indian seed and commercial raising regions, surveys were carried out every two weeks. From Bulgarian and Indian parents, two new breeding lines—the oval and dumbbell lines—were produced through the F3 generation. Six hybrids out of the 32 hybrids assessed in the artificial inoculation trial for root rot and root knot infections shown resistance to both diseases. Based on the results, two hybrid crosses with high production and better silk quality were found to be ICB14 x N23 and ICB17 x S8. Five pure breeds and two hybrids were purchased from Bulgaria in order to establish high producing silkworm breeds; they are now being assessed for further selection and use. In the South, East, and Northern parts of India, a multilocational study using a transgenic silkworm that is NPV resistant and was created via the RNAi method is now underway. NPV resistance was greater in transgenic silkworms than with the control group. A total of 35 hybrid combinations were selected and are now being

analysed in order to create silkworm hybrids that are acceptable for southern India's high temperatures and humidity levels. To choose the best silkworm for Eastern and North-Eastern India, 25 hybrids of high temperature and high humidity tolerance were raised under both normal and stressful circumstances.

For pebrine and NPV, an early detection technique based on PCR has been created. Through the introduction of NPV resistance, field testing of the three lines (MASN-4, 6 & 7) of NPV resistant CSR2 silkworms has begun under various agroclimatic conditions. Stakeholders are validating Loop-Mediated Isothermal Amplification (LAMP), a straightforward method for pebrine detection. A straightforward instrument for de-flossing cocoons was created and is now being evaluated for effectiveness. Prepared the foundation crosses and identified the appropriate breeding resource materials that are resistant of high temperatures and high humidity conditions. The planned raising of 473 silkworm germplasm stocks, including 81 Multivoltine, 369 Bivoltine, and 23 mutations, is being done. R&D initiatives have contributed to an increase in yield from 48 kg per 100 dfls in 2005–06 to 60.3 kg per 100 dfls in 2017–18.

Vanya Silk Research & Development: Vanya Host Plant

Found a fast-growing, easy-to-root alternative food plant, Lagerstroemia speciosa, for Tasar silkworm raising. The performance of raising is being tested. 10 superior Terminalia arjuna accessions (Accession Nos. 102, 115, 123, 135, 424, 507, 523, 525, 614, and 718) were chosen for additional screening in order to identify fast-growing, drought-tolerant accessions. Mucuna bracteata, a wild leguminous plant, Phosphate Solubilizing Bacteria (PSB), and tiny water catchments at 5 plantation sites make up the package devised to conserve moisture and replenish the soil with nutrients in the Terminalia plantation. Using this package, an average increase in leaf yield of up to 49.51% was observed. Two Som accessions (S3 and S6) that are resistant to rust, leaf blight, and leaf spot disease are becoming more and more common in the field. A package known as Integrated Nutrient Management (INM) was created for castor production and is now undergoing field testing. It has been determined that Ailanthus grandis (Barpat) is a possible perennial food source for Eri silkworms and that it should be used in the field. In comparison to Kesseru plants' leaf output of 25 MT/ha/yr, it recoded a leaf yield of 32 MT/ha/yr.

For the effective use of Sal flora in Jharkhand and to increase Laria production on Sal, a package of practises is advised. Castor and Alianthus grandis have comparable leaf biochemicals, according to biochemical research. The diseased castor leaves were used to isolate the Alternaria ricini in its purest form. Rhizobacteria isolates' antagonistic efficacies were examined in bioassay experiments, and isolates LRP-4 and HF-3 demonstrated the greatest pathogen inhibition. Ten rhizosphere soil samples from the Keonjhar area yielded a total of 64 PSB isolates, and work on estimating the PSB isolates' in vitro phosphate solubilization efficiency is ongoing.

Silkworm Vanya

'BDR-10' Tasar Daba bivoltine silkworms are becoming more well-known.Five different sites were used for a multi-location field experiment for the high fecundity Tasar silkworm line, CTR-14. In terms of productive qualities, an increase of 20–22% above the control group was seen.DTS and DT-12, two promising Tasar silkworm lines, were chosen, and 38250 of their seed cocoons are being preserved. The 'C2' Eri silkworm breed is becoming more well-known. CMR-1 and CMR-2, two improved Muga silkworm lines, are being field-tested. A

field test is being conducted on preservation regimens for Muga silkworm eggs designed to promote consistent hatching. Eri ecorace SR-025 is now undergoing a field experiment in the semi-arid region of Andhra Pradesh.

Antheraea frithi has been chosen as the potential future species of the NE area based on the characterisation, assessment, and classification of wild sericigenous insects. Based on body marking and colour, six potential eri silkworm strains—YP, YS, YZ, GBP, GBS, and GBZ—have been identified from the Borduar and Titabar ecoraces. Based on rearing results, two combinations—YZ x YS and GBS x GBZ—are identified as promising. There has been one successful experimental grainage of these mixtures. In four states—Assam, Meghalaya, Arunachal Pradesh, and BTC—the NERTPS initiative is being used to conserve Muga and other wild silk moth species in-situ.

Electroantennogram (EAG) was used to analyse the antennal responses of yellow fly throughout the feeding and spinning phases to volatiles isolated from the tasar host plant and Antheraea mylitta. Developed a natural module to combat muga silkworm illnesses and pests. With the use of comparative genomics, the pathobiome connected to the muga silkworm flacherie illness has been identified. The 68 to 72 hour old embryo represents the longest period of embryonic development, which aids in creating appropriate schedules for egg preservation. Pebrine visualisation solution field tests for light microscopy identification of pebrine spores were carried out at several BSMTCs and PPCs in Jharkhand, Odisha, Chhattisgarh, and Madhya Pradesh.

To boil Tasar cocoons more effectively, the chemical formula known as "Tasar Plus" has been created. Technologies created to dry Tasar cocoons with hot air utilising a conveyor hot air dryer. The spread of low-cost, eight-end, multi-end reeling equipment for tasar silk reeling. In the post-cocoon section of Vanya silk Tasar and Muga cocoon wet reeling, tasar silk sizing machines, modified dry reeling machines for tasar cocoons, pressurised hank degumming machines, and equipment for recycling silk reeling water are becoming more and more common in the industry.

Created a conveyor cocoon dryer with a 1.2 MT/day cocoon drying capability. Created the "Sonalika" reeling machine to replace the "Bhir reeling" of Muga cocoons. A demonstration of a machine that extracts the pellade layer from wasted silkworm pupae and separates the pupae. Developed and field-tested technique for "Use of Slug Catcher (as Replacement for Porcelain Button) for Slug Removing." Developed and field-tested technique for "Yarn degumming using CSTRI Eco degumming machine." The Institute's vertical reeling machine has been improved and transformed into a three-ends machine for increased output.

Developed fibroin matrix-reinforced Mulberry, Tasar, Muga, and Eri silk fabrics. Technology that uses the HTHP approach to extract sericin from silk yarn has been developed. Created several Chanderi saree variants (Silk x Silk). Created Mulberry single jersey shirts for women with digital prints. Mulberry interlock knits with tie-dye yarn, Mulberry and cotton union knits with dye variation as a design element, and Mulberry jacquard tuck knit patterns for women's shirts were developed. Eri silk nonwoven textiles have been successfully manufactured, and studies on impregnation with cosmetic formulations for face mask application are now underway at L'Oréal. Developed Knits composed of Mulberry & Cotton Mélange Yarns. Describing the sericin's use in cosmetics (soaps, shampoos, hair treatments, etc.) and talcum powder applications as addictive. Technologies for cooking Raily Tasar cocoons for wet reeling have been developed[11], [12].

CONCLUSION

The flacherie disease-related viral and bacterial pathogens in Antheraea mylitta D. were isolated and identified. Complete genome identification of the baulovirus that causes tiger band disease in Antheraea proylei (accession:GI 1371952746) In order to prevent the Bacillus species and Pseudomonas species-caused bacterial flacherie illness in the muga ecosystem, a novel chemical disinfectant has been developed, and bioassay investigations of the same are now being conducted in the lab. At JSDI Ranchi, PPC Kharsawa, PPC Hatgamaria, and PPC Bengabad (JH), RTRS Baripada (OD), RTRS Bhandara (MH), REC Kapista (WB), and REC Kathghora (CH), a trial of the semi-synthetic food "Tasar Amrit" was conducted. The availability of sericin in various fibre waste is around 1.8-2.5%, and it has been isolated and characterised from tasar silk fibre waste for commercial use. A total of 64 PSB isolates have been discovered in 10 rhizosphere soil samples from the Keonjhar area, and work is now being done to estimate how well these isolates swell with phosphate in vitro. To create superior grade import-substitute silk, R&D in Post Cocoon: Development & Demonstration of Indigenous Automatic Silk Reeling Machine (ARM). A demonstration of a solar-powered, reasonably priced spinning machine that may be used in rural settings.

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CHAPTER 2
COLLABORATIVE RESEARCH PROJECTS AND BIOMATERIAL RESEARCH

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ABSTRACT:
In addition to internally sponsored initiatives, the R&D institutions of CSB also conduct joint research projects with financial support from DBT, DST, MNRE, etc. A total of 17 research projects involving outside financing will be conducted in 2018–19.Other research institutes like IIT Kharagpur, IIT Guwahati, IARI New Delhi, IIHE Bangalore, CCMB Hyderabad, IISc Bangalore, BTRA Mumbai, GKVK Bangalore, ICARNBAIR Bangalore, NESAC Shillong, NEIST Jorhat, NBSS & LUP Jorhat, RFRI Jorhat, BIT Mesra, NCL Pune, etc. collaborate with CSB Institutes as well. Presently, partnership with several of these institutions is used to carry out 09 projects of this kind. There has also been international partnership with many institutions. A project on breed improvement is being carried out with a research institute in Bulgaria, and another project on the molecular characterization of the Ifla virus infecting the tasar silkworm with the Swedish Research Council has just been started. These projects are three with Deakin University in Australia for developing technology on post cocoon technology. A memorandum of understanding has been reached with research organisations in Australia, China, Japan, and Australia for the exchange of genetic material to enhance hybrid vigour. Training The R&D institutes of CSB are heavily active in training, skill seeding, and skill upgrading on a sustainable basis. These institutions are dispersed across the nation and cover all operations on the silk value-chain relevant to the four silk sub-sectors

KEYWORDS:
Development, Sericulture, Silk clusters, Technology, Training.

INTRODUCTION

The CSB has reorganised its capacity-building and training activities under the following five headings:

Programmes for Enterprise Development and Skill Training (STEP):

Numerous short-term training programmes with a focus on entrepreneurship development, internal and industry resource development, specialised overseas training, popularisation of sericulture technologies, lab to land technology demonstration programmes, training impact assessment surveys, etc. have been planned under this category. The Entrepreneurship Development Programme, Technology Upgrading Programme, Resource Development Program/Trainers Training Programme, Competency Enhancement Training Programme, Disciplinary Proceedings Training, Management Development Programme, etc. are some of the well-liked programmes under this component[1], [2].

Creation of Sericulture Resource Centres (SRCs):

These training and facilitation facilities, which were constructed in a few Mulberry, Bivoltine, and Vanya clusters at a cost of Rs. 2.00 lakhs each, serve as a vital conduit between the Extension Centres of R&D laboratories and the beneficiaries. These SRCs'

objectives include cluster-level issue solving, skill development, doubt clarification, and technology demonstration. They also serve as one-stop shops for Seri-inputs. 23 SRCs are active as of this writing[3], [4].

Capacity Building & Training by R&D Institutes of CSB:

For the purpose of empowering framers and other industry stakeholders, the R&D institutes of CSB also organise Krishi Melas, Farmer's Day, farmer's interaction workshops, etc. in addition to structured long-term training programmes (Post Graduate Diploma in Sericulture & Intensive Sericulture Training).

Strengthening Seed Sector Capacity:

The most important component driving the whole silk value chain is silkworm seed. The calibre of an industry's output is determined by the calibre of its seed.Therefore, it is crucial to address the sector's training and capacity development requirements. A range of training courses are being considered for industry participants such private seed producers for silkworms, adopted seed rearers, managers, and employees of government-owned grainages[5], [6].

Information, Education, and Communication (IEC):

IEC promotes suggested technologies via brochures, pamphlets, handouts, booklets, and other materials in order to assist capacity-building and training programmes. Additionally, this component plans to provide educational movies, study guides, and documentaries to showcase the sector.

Transfer of Technology (TOT):

Through a variety of extension communication programmes, including Krishimelas, Group Discussions, Enlightenment Programmes, Field Days, Farmers' Meets, Audio Visual Programmes, Technology Demonstrations, etc., the technologies that resulted from the completed projects are effectively transferred to the field. Up to the end of December 2018, 1195 ToT programmes had been planned for the 2018–19 academic year, and 45 technologies had been successfully transferred to the user level under the pre–cocoon sector. Additionally, 152 field programmes and technological demonstrations were carried out in the post-cocoon sector, and 83,322 cocoon and silk samples were analysed and the findings were presented. Implementing the cluster promotion programme for bivoltine silk is the first priority during the XII Plan, along with increasing domestic production of replacement silk to 5000 MT from the previous production level of 1985 MT (2012–2013). 172 Bivoltine Clusters have been organised by the Central Silk Board in collaboration with the State Sericulture Departments in order to meet the goal. In contrast to the country's aim of 6200 MT during 2017–18, 5874 MT of Bivoltine raw silk have been produced with the combined, focused efforts (a 11.5% increase over the 5266 MT produced in 2016–17). Out of the 5874MT total output of BV raw silk in the nation, bivoltine clusters produced 4100 MT [7], [8].

The Cluster Promotion Programme has been extended from 2017–18 to 2019–20 with a primary goal of achieving the country's objective of 8500 MT bivoltine raw silk production by the end of 2019–20. The number of clusters was reduced from 172 to 151 by restructuring/reorienting some of the existing clusters in the Northwestern area for effective monitoring purposes, without affecting the overall cluster aim. 4900 MT of bivoltine raw silk are predicted to be produced from 151 clusters in 2018–19, or around 68% of the entire

production goal of 7200 MT. The entire amount of BV raw silk produced in 2018–19 (from April to December 2018) was 4737 MT, with 2980 MT (63%) coming from clusters.

DISCUSSION

Cluster Promotion Programme for Vanya Silk Implementation:

Through the cluster strategy, the Central Silk Board has developed 22 vanya clusters in nine of the states that produce tasar in order to boost the Tasar silk industry. A total of 2792 recipients were served by this program's capacity building, exposure visits, door-to-door distribution of disinfectants, and awareness campaigns on technology transfer services. 1.997 lakh dfls in total were brushed by 960 Adopted Seed Rearers in the first seed crop, resulting in 70.89 lakh seed cocoons at a rate of 35.5 cocoons per dfl. These seed cocoons were turned into 7.57 lakh dfls by 165 private graineurs, of which 6.79 lakh dfls were raised by 2627 commercial farmers in the second crop (commercial), yielding 266.74 lakh cocoons at a rate of 39.72 cocoons per dfl in the clusters. Outside of the clusters, commercial farmers received the remaining 0.78 dfls.

JOCVs, or Japan Overseas Cooperation Volunteers:

Since 1991, CSB has launched many activities in collaboration with JICA to ensure the long-term viability of bivoltine in India. Under the first phase of JICA initiatives, CSB created Mulberry varieties, acceptable Bivoltine Breeds, and a full Bivoltine Sericulture Technology package that could be replicated in Indian conditions. During the second phase of the initiative, these technologies were field tested, and during the third phase, a thorough Extension System was created. The same is being replicated in India via CPP, and despite a goal of 5000 MT, 5200 MT of bivoltine silk were produced at the conclusion of the XII plan. Under the JICA Follow-up Cooperation Programme from 2012 to 2014, JICA has advised strict adherence to one-way seed multiplication for quality maintenance, adoption of JICA-recommended technology for race maintenance to preserve the race's characteristics, and strictly adherence to Rotary Mountages Technology with net collection techniques for quality silk production. An indigenously built automatic reeling machine has also been constructed on the Post Cocoon Sector with the assistance of JICA, and attempts have been undertaken to enhance it by the addition of a Harada water ejection system [9], [10].

Technology initiatives

DBT MIS: STQC has approved the security audit of the DBT MIS that was developed for the "Development of Silk Industry" project. mKisanCSB has expanded the reach of scientists and specialists to communicate information so that farmers may get scientific recommendations through their mobile phones. Through this site, advisories are often provided by all the major institutions. 11,15,000 SMS messages have been issued as part of 320 alerts thus far. 'SMS service' through mobile phone on current market prices for silk and cocoons for use by farmers and other industry players. There are active PUSH and PULL SMS services. All 8517 enrolled farmers are getting daily SMS messages at their registered mobile numbers, which DOS has updated.

SILKS Portal:

The Sericulture Information Linkages and Knowledge System portal was created in collaboration with the North Eastern Space Application Centre, Department of Space. It uses satellite-based geographic image analysis to identify potential locations for promoting sericulture activities. Data that spans many districts and languages is updated often.

Video Conference:

The CSB Complex in Bangalore, CSR&TI in Mysore & Berhampore, CTR&TI in Ranchi, CSR&TI in Pampore, CMER&TI in Lahdoigarh, and RO in New Delhi all have full-fledged video conference facilities. There are 26 multi-studio video conferences held from April 2018 to December 2018.Website of the Central Silk Board is a bilingual English and Hindi version of the website. For the benefit of the average person, who may need to know about the organisation as well as plans and other data, the most information possible is given via this page. The website highlights sericulture plan programmes, accomplishments, and sharing of success stories. A GIGW compliance and security assessment of the new CSB website is now being performed in accordance with government of India regulations.

AEBAS:

Central Silk Board is implementing a biometric attendance system with Aadhaar support. Farm labourers are among the 4254 employees who have registered on the attendance site. The 121 devices have all been made RD Services-capable.

National Farmers and Reelers Database:

This site was created and planned to hold a national database of Farmers and Reelers, which would provide policymakers with relevant data for efficient decision-making. As of December 30, 2018, the states have entered 6,61,427 farmers' and 10493 reelers' data into the database. For trouble-free access by all stakeholders, MIS for Intensive Bivoltine Sericulture is created and hosted on dedicated servers.

BPO for communicating with FRDB farmers:

Each zone's nodal officials communicate with a limited group of farmers while regularly acquiring their cellphone numbers from the FRDB database.

Mobile App Development for Silk Samagra: Data collection and design are ongoing processes.

Seed Organization

Basic seeds are supplied to the States via a system of Basic Seed Farms owned by the CSB.

Its commercial seed production facilities support U.S. attempts to provide farmers with commercial silkworm seed.

Vanya Silk, which is made from the cocoons of wild silkworms, is a priceless and resilient natural fibre with enormous potential for improving rural lives and stimulating the economy. Small, marginalised people in wooded areas of India and other nations produce the majority of the world's Vanya Silk. Implementing a Cluster Promotion Programme (CPP) particularly designed for this industry is crucial to maximising the economic potential of Vanya Silk and enhancing the living circumstances of these people. A CPP is a planned strategy for cluster development that attempts to make SMEs more competitive in a particular industry or area. To encourage sustainable and inclusive development in the instance of Vanya Silk, a CPP would bring together a variety of stakeholders, including silk growers, weavers, merchants, and governmental organisations. The main procedures and elements of a CPP for Vanya Silk implementation are described in this manual.

Cluster Recognition

Identifying and mapping the current silk manufacturing clusters is the first stage in putting a CPP for Vanya Silk into action. Clusters are geographical gatherings of associated companies and organisations operating in a certain industry. Clusters may include villages that produce silk, weaving communities, and commercial centres in the context of Vanya Silk. Effective resource and intervention targeting is made possible by cluster identification The essential steps in cluster identification are:

a. **Assessment and Mapping:**

To locate existing clusters, conduct a thorough assessment of the Vanya Silk producing regions. For precise mapping, make use of remote sensing and geographic information systems (GIS).

b. **Stakeholder Consultation:**

Talk to local residents, silk producers, weavers, and other interested parties to learn more about the whereabouts and dimensions of clusters.

c. **Data Analysis:**

Examine the data gathered to identify any probable clusters based on the volume of actions using silk.

d. **Cluster Profiling:**

Create profiles for each detected cluster that include the quantity of production, the number of families engaged, and the main difficulties.

Increasing Capacity

Enhancing the capabilities of people and businesses within selected clusters is the next stage. Building capacity is crucial for raising the calibre and output of Vanya Silk weaving and manufacturing.

Principal exercises in capacity building:

.Training Programmes: Set up educational opportunities for silk growers and weavers to learn about sericulture, cocoon care, silk extraction, and weaving methods.Promote the implementation of cutting-edge sericulture techniques that are sustainable, such better mulberry cultivation and disease control.

Design and Innovation:

Promote creativity in the design and development of silk products to meet shifting consumer expectations.

Entrepreneurship Development:

Train weavers and manufacturers of silk in entrepreneurship and business management so they may run their businesses successfully.

Development of the Market and Coordination.

Board Secretariat, Regional Offices, Certification Centres, and Raw Material Banks are all part of the central Silk Board administration. The CSB Board Secretariat collaborates with

the Ministry and States to execute different initiatives in the sericulture sector while keeping an eye on the execution of various schemes. The Board Secretariat organises several national meetings, board meetings, review meetings, and other important gatherings. The Raw Material Banks use a floor price to control the market price of cocoons and guarantee that primary producers are paid fairly. The main goal of the Tasar Raw Material Bank (RMB) in Chaibasa, Jharkhand, and the Muga Raw Material Bank (MRMB), which is currently merged with the Regional Sericulture Research Station (RSRS), Jorhat, Assam, is to ensure an equitable and reasonable price for the actual Tasar & Muga cocoon producers.

We can better understand biomaterials, their characteristics, and their uses in a variety of sectors, such as medicine, tissue engineering, and biotechnology, by working together on collaborative research initiatives. These initiatives often include multidisciplinary teams of researchers, engineers, and medical professionals working together to solve complicated problems using cutting-edge biomaterials. An overview of joint biomaterials research initiatives is provided below: Teams from several disciplines, such as materials science, biology, chemistry, engineering, and medicine, are often involved in collaborative biomaterial research initiatives. These teams combine complementary expertise and talents to address challenging research topics.

Research Goals:

While the precise goals of collaborative biomaterial research initiatives might vary greatly, they often centre on the creation and assessment of biomaterials for particular uses. Examples include creating tissue scaffolds, medication delivery systems, or implants using biocompatible materials. Research teams collaborate to develop, create, and perfect biomaterials with the needed qualities. In order to improve a material's biocompatibility, mechanical strength, or other properties, this may include creating new materials or altering existing ones. Biomaterials need to work with biological systems without causing any harm. In order to make sure that biomaterials do not cause unfavourable immunological reactions or harmful consequences, collaborative initiatives often examine the interactions between biomaterials and live tissues.

Tissue engineering:

Some group efforts concentrate on employing biomaterials to develop synthetic organs or tissues. Researchers are working to create scaffolds that encourage cell differentiation and development, eventually producing viable tissue substitutes.

Drug Delivery Systems:

Controlled drug delivery systems may be made using biomaterials. In order to increase the effectiveness and safety of therapies, collaborative efforts may include creating carriers that release medications at certain rates or places throughout the body.

Characterization and Testing:

To characterise biomaterials, collaborative research teams use a range of analytical methods, including spectroscopy, microscopy, mechanical testing, in vitro and in vivo investigations. The materials are better able to fulfil the needs they were designed to.

Clinical Translation:

The goal of many joint biomaterial research collaborations is to move discoveries made in the lab to use in patients. To satisfy safety and effectiveness criteria, this often entails close collaboration with regulatory bodies and healthcare practitioners.

Finance and Resources:

Collaborative initiatives often need a significant amount of finance as well as access to specialised facilities and equipment. To fund their study, researchers may apply for funding from governmental organisations, private foundations, or business partners.

Data Sharing and Communication:

In a collaborative research project, team members must effectively communicate and share data. Sharing results, developments, and difficulties may assist the project go ahead and provide positive results. Biomaterials-related collaborative research initiatives have the potential to significantly advance the disciplines of medicine, biotechnology, and other industries. They enable researchers to combine their knowledge and resources to tackle difficult problems, which results in the creation of cutting-edge biomaterials and technologies that may enhance human health and quality of life[11], [12].

CONCLUSION

Efforts have been made to introduce Community Based Organisations (CBOs) and Self Help Groups (SGHs) among the farmers in order to carry out knowledge and technology transfer among the farmers, in addition to the existing extension activities, such as group activities, credit facilitation, thrift and better price realisations, for the sustainable growth of Bivoltine silk. For three years starting in January 2015, JICA has delegated three Japan Overseas Cooperation Volunteers (JOCV) to operate in collaboration with CSB/DoSs CDFs in 10 clusters (8 in Karnataka, Andhra Pradesh, and Tamil Nadu, and two in Uttarakhand). The major goal of JOCVs is to aid CSB/State Counterparts in Bivoltine Clusters in identifying field issues and to help Extension technique in setting up Self Help Groups/CBOs including sericulturists for efficient technology transfer designated Clusters.

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CHAPTER 3
PRODUCT DESIGN, DEVELOPMENT AND DIVERSIFICATION

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ABSTRACT:

Product creation, design, and diversification are essential steps for businesses looking to succeed in today's fast-paced, cutthroat corporate environment. This article examines the relevance of these related ideas, emphasizing their guiding principles, difficulties, and ideal practices. The revolutionary power of good product design, development, and diversification is shown via case studies of prosperous businesses like Apple and Amazon. When these tactics are used strategically and with the consumer in mind, firms are able to design goods that customers want to buy, expedite the development process, and enter new markets. In conclusion, businesses that want to survive and thrive in the constantly changing business environment must adopt this comprehensive approach to innovation.

KEYWORDS:
Design, Development, Goods Silk, Product

INTRODUCTION

The P3D activities are to give special focus on fabric engineering, silk blends, designing new fabric structures, designing and developing new products in silk and silk blends, product development in the clusters, 14 commercialization of developed products, helping the commercializing partners in providing backward linkage, technical know-how, and helping/coordinating in sample development[1], [2].

P3D's activities Resurrection of Traditional Silk Goods, Design innovation and product diversity using mixes, the creation of products based on a set of specified preferences and requirements for both its design and intended usage. Producing market data, updating market data, and predicting vogue trends.Themed pavilions and product displays at silk expos and exhibits to promote Indian silks generally and by brand.Assist silk exporters and manufacturers in creating cutting-edge textiles and patterns that meet consumer desire.A showcase of the most recent advancements in silk goods, with the ultimate goal of establishing a Centre of Excellence for Indian Silk advancements.Products Developed:

1. Power loomed Muga Satin fabric and clothing

2. Eri and Mulberry knits, Eri silk blanket and carpet, and Eri silk thermal wear. Eri silk denim materials for blazer and clothing.

3. Tasar silk fabric for wedding gown on electric looms.

4. Fabrics in the Chanderi cluster and pure silk sarees

5. Muga silk is used in lieu of zari in Kanchipuram sarees.

6. Aroma-treated and stain-guard sarees

7. Silk lifestyle items for women, including handbags, socks, gowns, and accessories

8. Fabrics and silk saris printed in the Bagh (MP) cluster

9. Items with authentic Lambani artwork

10. Sarees by Mulberry X Eri with Bomkai Design

11. Mulberry saree with a tribal design from Nagaland and textiles made of silk, linen, cotton, and modal

The Quality Certification System's ability to increase quality assurance, quality evaluation, and quality certification is one of its primary goals. The "Cocoon and Raw Silk Testing Units" and "Promotion of Silk Mark" components of the strategy are now being put into action. The performance during reeling and the quality of the generated raw silk are influenced by the quality of the cocoons. Cocoon testing is facilitated by cocoon testing centres that have been created in several cocoon markets with CDP funding. To assure the quality of the silk products exported from India, the Central Silk Board's network of Certification Centres connected to the Regional Office performs voluntary pre-shipment inspections of silk items intended for export.Additionally, the Silk Mark Organisation of India (SMOI), a subsidiary of the Central Silk Board, is promoting "Silk Mark" as a symbol of the purity of silk goods. A guarantee label called "Silk Mark" defends customer rights against merchants who market fake silk items as genuine[3], [4].

Expos for Silk Mark Silk Mark Expos are being organised solely for Silk Mark Authorised Users from all around the nation in order to guarantee that Silk Mark continues to develop legitimacy and popularity. The Expo is the perfect venue for promoting Silk Mark as well as uniting producers and customers to facilitate the sale and purchase of goods made of pure silk. During this event, the participants earn a significant amount of business. The SMOI runs extensive awareness and publicity activities during the event.Due to the weak demand for silk goods and the unenthusiastic reaction from the Authorised Users, SMOI arranged four Silk Mark Expos throughout the 2018–19 year (from April to December 2018) in the following locations.Authorised Users have recently been hesitant to participate in Silk Mark Expos owing to increasing stall rent, thus SMOI is looking into the possibility of holding special handloom Expos in conjunction with the Development Commissioner (Handlooms) to make up for this. For three expos in Pune, Hyderabad, and Vizag, financial support has been granted in principle by DC(H).More steps are being made to investigate the viability of hosting Expos in other cities during the next fiscal year.The "National Level Special Handloom Expo" was held at the Hotel Green Park in Vishakhapatnam from November 21 to December 4, 2018—a total of 14 days. It was conducted by the SMOI, Hyderabad Chapter. The Development Commissioner for Handlooms, Ministry of Textiles, Government of India, provided funding for this silk expo[5], [6].

1. From May 21 to May 27, 2018, the Kerala state government held "NAVKERALAM 2018," a spectacular exhibition at the Palakkad stadium grounds. The SMOI Palakkad branch set up a theme pavilion with 100 square feet of booth area to exhibit all the operations in the Silk chain, from the soil to final goods.

2. To promote and raise awareness of Silk Mark among customers, the Hyderabad chapter held the Curtain Raiser of Srimathi Silk Mark 2018 on the grounds of the Department of Sericulture at Jubille Hills, Hyderabad.

3. The great sixth edition of the Srimathi Silk Mark-2018 was held on April 21, 2018, at Kalinga Hall in Banjara Hills, Hyderabad, by the SMOI, Hyderabad branch. More than 400

individuals from all walks of life, including members of the general public, NIFT students and teachers, ATDC staff, CSB officials, and notable public and private figures, have come to the massive event. Models walked down a ramp while performing a cultural presentation that showcased the recently produced silk goods by P3D and VSMPC.

4. Due to the overwhelming positive feedback received from Resham Ghar, A Home of Pure Indian Silks, which was launched on an experimental basis in New Delhi in partnership with Lepakshi, a second "Resham Ghar" showroom has been established at M.G.Road in Bengaluru in the facilities of M/s. Central Cottage Industries Corporation (CCIC).

In the Resham Ghar @ CCIC, six authorised Silk Mark users from Karnataka, Telangana, Jharkhand, West Bengal, New Delhi, and Assam have been given stall space. Resham Ghars are being established by SMOI and CCIC in Chennai and Kolkata, respectively.

5. From August 4 through August 15 for 12 days, the Bangalore branch of SMOI will host a Flower Show at the Glass House in Lalbagh, Bangalore. For the marketing of Silk Mark, a theme pavilion with three Silk Mark logos, Silk Mark India, and a silk moth emerging from a cocoon was on show.

6. To raise awareness among the general public regarding the use of biodegradable containers for everyday use, SMOI Corporate Office staged three street plays as part of the Swachata Abhiyan, one at Lalbagh in Bangalore on September 29 and the other at Cubbon Park on September 30, 2018.

7. To build a brand image for the Indian silk industry and in partnership with RBSM, the Indian Silk Export Promotion Council, New Delhi organised the 6th India International Silk Fair at Pragati Maidan, New Delhi from October 16 to 18, 2018, in order to give entrepreneurs the chance to showcase their goods. Smriti Zubin Irani, a Our "Silks of India" pavilion was opened by the honourable union textile minister, who also used the opportunity to unveil a look book featuring NE silk goods. Other ISEPC dignitaries, High Commissioners from Japan and Korea, and the Joint Secretary (Silk) were also present.

8. On October 22, 2018, the Tie and Dye Handloom Silk Sarees Producers Association of Bhoodan Pochampally and Pochempally Handloom Park, Pochempally hosted an Interaction Programme on Silk Product Development and Commercialization. A number of Authorised Users of Silk Mark and weavers attended.

The attendees were informed by SMOI of the significance of product innovation and diversification, as well as the many beneficiary-oriented programmes being conducted in the state under several sectors, in particular

Application of "Silk Samagra" Integrated Scheme for Development of Silk Industry (ISDSI) Components for Loom Upgrade.

9. "Silk Composite Workshop at Lucknow": On December 22, 2018, at the Sugarcane Farmer Institute at Dolly Ganj, Lucknow, SMOI, Varanasi chapter took part in the "Silk Composite Workshop and P. Dinadayal Upadhyaya Silk Productivity Award Programme." The Central Silk Board and the Directorate of Sericulture, both under the control of the Uttar Pradesh government, together staged this event. The workshop was opened by the honourable Minister of State for Textile of the Government of India, Shri Ajay Tamta, who also recognised silk farmers.

10. Bhubaneshwar's 13th Toshali Mela: SMOI participated in the 13th Toshali National Crafts Mela, which was held at Janata Maidan in Bhubaneshwar, Odisha from December 28, 2018, to January 13, 2019, and was arranged by the Handloom, Textiles and Handicrafts Department of the Government of Odisha. Eri Silk knits, Tasar silk peduncle textiles, sarees, made-ups, and other silk items were shown in a theme pavilion set up by SMOI. Silk Mark labelled products and Silk Mark, CSB activities were also emphasised.The SMOI New Delhi Chapter took part in the Trade Enclave/Workshop on "Khadi- Batik Weave Going Global" held on December 18 at the Indonesian Embassy in New Delhi. The event's goal was to examine the potential for design collaboration between India and Indonesia as well as to create cutting-edge new designs and structures to satisfy the demands of burgeoning international markets. KVIC, IIFT, and FICCI sponsored the event in collaboration with the Indonesian Embassy in New Delhi.

A. Convergence activities:

The sericulture industry in the UK is receiving assistance from the Ministry of Textiles.form of NERTPS and CSS. Additionally, efforts are being conducted to mobilise more funding through convergence, by taking advantage of the programmes being implemented by numerous other Indian government ministries. According to the most recent information from States, during the 2017–18 fiscal year, opposed the requests for Rs. 933.86 crores.Because the States have been granted permission to spend Rs. 797.00 crores, of which Rs. for a variety of sericulture-related initiatives under RKVY, crores have been released[7], [8].

Mahila Kisan Sashaktikaran Pariyojana

Using the effective models created under unique SGSY Projects in for replication, CSB and MoRD took on multi-state in Bihar and Jharkhand projects involving the Government of Andhra's Society for the Elimination of Rural Poverty Bihar Rural Livelihood Promotion Society (BRLPS), the government of Bihar (SERP), Mahila Kisan, Bihar, PRADAN, BAIF, and Kovel Foundation Non-Timber Forest Produce (NTFP), also known as Sashatikaran Pariyojana (MKSP), is a the National Rural Livelihoods Mission's (NRLM) subsidiary. The seven initiatives Consider covering 23 districts' worth of 36117 Mahila Kisans (26094 in the Tasar sector) across 8 states at a total cost of Rs. 71.60 crore, split 75:25 between MoRD and CSB. The initiative aims to rejuvenate 9468 acres and raise 3503 hectares of tasar host flora establishing capabilities to manufacture 6.75 lakh dfls of basic materials from natural tasar flora commercial seed, 59.35 lakh dfls seed, and 16.09 billion reeling cocoons in addition 478 CRPs are being nurtured for up-scaling activities[9], [10].

Organisations must constantly innovate to be competitive and suit the shifting requirements and preferences of consumers in the quickly changing business environment of today. This innovation process requires the creation, development, and diversity of products. This article examines the relevance of these three interrelated ideas, including their main ideas, difficulties, and successful strategies.

Product design

The Value of Good Product Design

The first stage in producing a successful product is product design. It includes a product's appearance, usability, and user experience. In addition to drawing in consumers, a well-designed product improves both its marketability and use. Important aspects of product design include:

Aesthetics:

A product's aesthetic attractiveness may provide a positive first impression and set it apart from rivals**.**

Functionality:

The product must successfully and efficiently serve its intended function**.**

User Experience (UX):

Design should put the needs of the user and usability first.

Product Design Principles

Adherence to a number of basic principles is necessary for effective product design.

User-Centered Design:

Throughout the design process, keep the requirements and preferences of the end users in mind.

Simplicity:

Make the design as straightforward as you can without losing usability or appeal.

Consistency:

Consistently apply the same design language to every part of the product.

Sustainability:

Take into account how materials and production methods affect the environment.

Product Design Challenges

Product design is important, but it also presents a number of difficulties:

Rapid technological advancements might be difficult to keep up with, yet doing so is necessary to remain competitive.

Budget Restraints:

It might be challenging to strike a balance between superior design and financial constraints.

Changing Consumer Preferences:

Adapting to changing consumer preferences calls for constant change.

The Process of Product Development

The product enters the development stage once it has been designed. Making an idea into a concrete product that is ready for market launch is called product development. Key actions consist of:

Conceptualization:

Identifying the function, characteristics, and target market of the product.

Prototyping:

Building a working prototype of the product to test and improve it.

Testing:

Getting input, adjusting as needed, and assuring quality.

Manufacturing:

Increasing output to meet demand.

Agile Development

Agile development approaches are becoming more and more popular because of their adaptability and flexibility. These guidelines support cross-functional team cooperation, iterative development, and a customer-centered approach. Agile development improves product quality while reducing time to market[11], [12].

CONCLUSION

Product design, development, and diversification provide the groundwork for long-term growth and competitiveness in today's fast-paced corporate climate. Organisations may develop visually beautiful, useful, and user-centric goods that appeal to consumers via excellent product design. When the product development process is done with agility and consumer input in mind, these items are certain to reach the market quickly while maintaining high standards of quality. By lowering risk, extending market reach, and remaining one step ahead of the competition, diversification gives a strategic edge, as shown by successful businesses like Apple and Amazon. It does, however, have its own set of difficulties that need careful planning and resource allocation. In conclusion, for businesses looking for long-term success, integrating product design, development, and diversification is crucial. Organisations may adjust to changing customer tastes, negotiate technology improvements, and capture opportunities in new markets by adopting these ideas and methods. Businesses may not only prosper but also lead their respective sectors by placing consumers at the centre of these processes and building an innovation culture.

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CHAPTER 4
FUNCTIONING OF CENTRAL SILK BOARD

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ABSTRACT:

Through creative methods, the Research & Training Institutes of the CSB encourage scientific and technical advancements in output and productivity for sustainable sericulture. Mulberry sericulture is the primary focus of the major institutions in Mysore (Karnataka), Berhampore (West Bengal), and Pampore (J&K), while Tasar culture is the focus of Ranchi (Jharkhand), Muga and Eri culture is the focus of Lahdoigarh, Jorhat (Assam), and Ranchi (Jharkhand) institutes. Regional Sericulture Research Stations have been operating to build a technological package appropriate to the area and disseminate research results such as needed by the area. Additionally, a network of Research Extension Centres (RECs) and its subunits seek to help sericulturists in their extension activity. The Board has created a Central Silk Technological Research Institute in Bangalore to boost R&D in the post-cocoon industry. The CSB has also established the Central Sericultural Germplasm Resource Centre at Hosur (Tamil Nadu), the Seri-Biotech Research Laboratory in Bangalore, and the Silkworm Seed Technology Laboratory in Bangalore (Karnataka).

KEYWORDS:
Field,Mulberry, Silk, Silkworm, Taser.

INTRODUCTION

Act No. LXI of 1948, an Act of Parliament, created the Central Silk Board (CSB), a Statutory Body, in 1948. It operates under the administrative supervision of the Government of India's Ministry of Textiles, which is headquartered in Bangalore. The Board is made up of 39 individuals who were chosen for three years in accordance with the authority granted by Sub-Section 3 of Section 4 of the CSB Act 1948. The Central Government will appoint the Board's Vice-Chairperson and Secretary, both of whom must be officers with at least the rank of Joint Secretary to the Government. The Central Government will also nominate the Chairperson of the Board and two other officials, one of whom will be the head of the Silk Division in the Ministry of Textiles.The Central Silk Board has established 8 Regional Offices in New Delhi, Mumbai, Kolkata, Hyderabad, Bhubaneshwar, Guwahati, Lucknow, and Patna in order to coordinate the sericulture development initiatives in various States and to carry out pre-shipment inspection of silk items intended for export. To coordinate the transmission of technology, Regional Offices of CSB work closely with State Sericulture Departments, field units, and CSB field functionaries. Regional Offices also organise meetings of the Central Silk Board's State Level Sericulture Coordination Committee. As of January 1st 2019, CSB has 2,781 employees on staff[1], [2].

The CSB's mandated activities include research and development, upkeep of a four-tier network for the production of silkworm seeds, leadership in commercial production of silkworm seeds, standardisation and implementation of quality standards in various production processes, and advice to the government on all issues relating to sericulture and the silk industry. Through an integrated Central Sector plan called "Silk Samagra," an integrated plan for the development of the silk industry, the 192 units of the Central Silk

Board are carrying out these specified operations of the CSB. Silk Samagra has the following four components:

1.Research & Development, Training, Transfer of Technology and I.T. initiatives.

2.Organization Seed.

3.Coordination and the growth of the market.

4.Systems for quality certification, export, brand promotion, and technology advancement.

IT initiatives, training, transfer of technology, and research & development

The various R&D institutes of CSB have started a total of 16 new research projects during the 2018–19 academic year, and 30 projects have been completed as of the end of December 2018. Currently, a total of 98 research projects, including 58 in the Mulberry Sector, 30 in the Vanya Sector, and 10 in the Post Cocoon Sector, are in various stages of completion. Highlights of Research Programmes in Research & Development R&D on the host plant, the mulberry The commercial exploitation of the varieties G4 for the South, C2038 for the East and North regions, and Tr-23 for the mountainous parts of the East and North-Eastern zone. Found three genotypes of mulberry with high leaf production and tolerance to low temperature stress: C-108 (15.4 mt), C-384 (9.7 mt), and C-212 (9.2 mt). With leaf yields of 49.9 & 34.0 t/ha/yr under full & half of NPK dosage above 44.1 & 26.8 t/ha/yr of S-1635 variety in West Bengal Condition, the developed mulberry hybrid C-9 is appropriate for low input conditions. In West Bengal, Assam, and other Eastern and North Eastern States, the mulberry cultivar C-2028, which can withstand standing water, is becoming more and more well-known. Under the circumstances of West Bengal, five genotypes—C-01, C-384, C-11, C-02, and C-05—were shown to have superior growth characteristics and leaf yield to the control variety S-1635. AGB-8, PPR-1, and C1360 (Ganga), three novel mulberry cultivars, have been selected for testing in the subsequent stage of the All India Coordinated Experimental Trials for Mulberry (AICEM)[3], [4].

Found three potential genes for cold tolerance that might be used to create cold-tolerant mulberry variants for temperate locations. The leaf roller (Diaphania pulverulentalis) male insects may be caught using the pheromone chemicals Z-11 hexadecenal, Octadecane, and Z-e-7, 11- hexadecenal acetate. EAG is currently being developed, along with the pheromone molecule. In order to amplify the genes for Nitrogen Reductase and Chalconne Synthase, DNA from 110 progeny was taken. The induction of callus from the fusion of four different mulberry variety protoplasts. Vishala, a legal kind of mulberry, is now gaining popularity in Eastern and North-Eastern regions. In West Bengal, under irrigated circumstances, Vishala recorded a 12.21% greater leaf yield, or 10267 kg/ha/crop, above S1635, or 9150 kg/ha/crop. There were issued 17236 soil health cards.By adding 55 additional collections, the number of mulberry accession being conserved at CSGRC Hosur has climbed to 1291. With an effectiveness of 88-94% disease suppression, a novel formulation called "Rot fix" was created to treat root rot. Ankur, a mix of organic and inorganic nutrients, was advised since it increased cocoon and mulberry leaf yields by 11% and 14%, respectively [5], [6].

DISCUSSION

A mulberry pest incidence calendar for various agro-climates in the Eastern and North Eastern areas was established in order to control mulberry pests effectively. In the field, the bio-nematicide "Nemahari" reduced root knot disease by up to 80% while improving leaf production by 15% to 18%. Investigated the prevalence of root rot infections in Kashmir

areas and determined that Helicobasidium mompa and Fusarium oxiforum were the responsible organisms. The field performance of mulberries at various spacings revealed that the best production occurred at (150+90) cm x 60 cm (13174.83 kg/ha/crop), while the lowest yield occurred at 270 cm × 60 cm (8842.17 kg/ha/crop). Mulberry productivity increased from 50 MT/Ha/yr in 2005–2006 to 60 MT/Ha/yr in 2017–18 because to R&D initiatives.

R&D on the Mulberry Silkworm

The Hybrid Authorization Committee proposed the approval of two novel bivoltine hybrids, G11xG19 and B.con1 x B.con4, with cocoon yields of 68.5kg/100 dfls and 58kg/100 dfls, respectively. The procedure of notifying the Gazette is now underway. A large-scale, multi-location study including MV1xS8 and S8x CSR16 is now being conducted at the farmer level. For a small number of field tests, N21 x N56, a thermo-tolerant bivoltine double hybrid with a yield potential of 72 kg/100 dfls & 21.5% shell, was chosen. Using SSR markers (LFL0329 & LFL1133) related to thermo-tolerance, four thermo-tolerant silkworm lines were created.Two bivoltine hybrids, CSR52N x CSR26N and (CSR52NxS8N) x (CSR16NxCSR26N), that are NPV-tolerant have been shortlisted for field testing. To test the potential for yield and thermotolerance, two enhanced crossbreeds—L3 x S8 and HB4 x S8—that are resistant to high temperatures and BmNPV, have >90% pupation rates, 20–21% shell ratios, and 14–15% raw silk—were chosen for field trials. Four hybrids were chosen for fall raising in North India: CSR46 APS9; APS9 BBE 198; APS5 APS9; and SK6 SK7[7], [8].

Through shuttle breeding, two novel bivoltine single hybrids, BHP-2 x BHP-8 and BHP-3 x BHP-8, were created with greater shell percentages than the current hybrids. For the Eastern area, novel bivoltine silkworm hybrids Gen-3 x SK6 and M6DPC x (SK6 x SK7) with cocoon production potential of 50–55 kg yield/100 dfls and 45–50 kg yield/100 dfls, respectively, were produced. CSR4 and CSR27 NPV resistant lines were created by introducing the RNAi mechanism from the transgenic silkworm Nistari. These CSR4 and CSR27 lines demonstrated greater NPV resistance.

Three breeds of silkworm, including APS-5, APS-HTP5, and BBE198, were determined to be DNV resistant based on the presence or lack of the nsd-2 DNV resistant gene. There are five breeds: M.Con.4Id, M.Con.4Id, B.Con.4Id, and BHB Id constructed using Id characters. In Pichia pastoris, a full-length lipoprotein gene was created and cloned for the extraction and characterisation of recombinant proteins. To track the prevalence of illness in South Indian seed and commercial raising regions, surveys were carried out every two weeks.

From Bulgarian and Indian parents, two new breeding lines—the oval and dumbbell lines—were produced through the F3 generation. Six hybrids out of 32 hybrids tested in an artificial inoculation research for root rot and root knot infections shown resistance to both diseases.Based on the results, two hybrid crosses with high production and better silk quality were found to be ICB14 x N23 and ICB17 x S8.Five pure breeds and two hybrids were bought from Bulgaria in order to create high producing silkworm breeds; they are now being tested for use and further selection. In the South, East, and Northern parts of India, a multilocational study using a transgenic silkworm that is NPV resistant and was created via the RNAi method is now underway. In comparison to the control, transgenic silkworms had greater levels of NPV resistance. A total of 35 hybrid combinations were selected and are now being assessed in order to create silkworm hybrids that are acceptable for southern India's high temperatures and humidity levels. To choose the best silkworm for Eastern and North-Eastern India, 25 hybrids of high temperature and high humidity tolerance were raised under both normal and stressful circumstances[9], [10].

For pebrine and NPV, an early detection technique based on PCR has been created. Through the introduction of NPV resistance, field testing of the three lines (MASN-4, 6 & 7) of NPV resistant CSR2 silkworms has begun under various agroclimatic conditions. Stakeholders are validating Loop-Mediated Isothermal Amplification (LAMP), a straightforward method for pebrine detection. A straightforward instrument for de-flossing cocoons was created and is now being evaluated for effectiveness. Prepared the foundation crosses and identified the appropriate breeding resource materials that are resistant of high temperatures and high humidity conditions. The planned raising of 473 silkworm germplasm stocks, including 81 Multivoltine, 369 Bivoltine, and 23 mutations, is being done. R&D initiatives have contributed to an increase in yield from 48 kg per 100 dfls in 2005–06 to 60.3 kg per 100 dfls in 2017–18.

R&D on Vinay Silk

Found a fast-growing, easy-to-root alternative food plant, Lagerstroemia speciosa, for Tasar silkworm raising. The performance of raising is being tested. 10 superior Terminalia arjuna accessions (Accession Nos. 102, 115, 123, 135, 424, 507, 523, 525, 614, and 718) were chosen for additional screening in order to identify fast-growing, drought-tolerant accessions. A strategy was created to include tiny water catchments at Terminalia plantations in order to conserve moisture and enrich the soil with nutrients. Site of a plantation, Mucuna bracteata, a wild legume, and phosphate-solvent bacteria (PSB). Using this package, an average increase in leaf yield of up to 49.51% was observed.

Two Som accessions (S3 and S6) that are resistant to rust, leaf blight, and leaf spot disease are becoming more and more common in the field. A package known as Integrated Nutrient Management (INM) was created for castor production and is now undergoing field testing. It has been determined that Ailanthus grandis (Barpat) is a possible perennial food source for Eri silkworms and that it should be used in the field. Compared to Kesseru plants' leaf output of 25 MT, it recoded a leaf yield of 32 MT/ha/yr. For the effective use of Sal flora in Jharkhand and to increase Laria production on Sal, a package of practises is advised.

Biochemical testing revealed similarities between Castor and Alianthus grandis leaves. The diseased castor leaves were used to isolate the Alternaria ricini in its purest form. Rhizobacteria isolates' antagonistic efficacies were examined in bioassay experiments, and isolates LRP-4 and HF-3 demonstrated the greatest pathogen inhibition. Ten rhizosphere soil samples from the Keonjhar area yielded a total of 64 PSB isolates; work is now being done to estimate the effectiveness of their in vitro phosphate solubilization.

Silkworm Vinay

'BDR-10' Tasar Daba bivoltine silkworms are becoming more well-known.Five different sites were used for a multi-location field experiment for the high fecundity Tasar silkworm line,CTR-14. In terms of productive qualities, an increase of 20–22% above the control group was seen.DTS and DT-12, two promising Tasar silkworm lines, were chosen, and 38250 of their seed cocoons are being preserved.The 'C2' Eri silkworm breed is becoming more well-known.CMR-1 and CMR-2, two improved Muga silkworm lines, are being field-tested.A field test is being conducted on preservation regimens for Muga silkworm eggs designed to promote consistent hatching.Eri ecorace SR-025 is now undergoing a field experiment in the semi-arid region of Andhra Pradesh.Antheraea frithi has been chosen as the potential future species of the NE area based on the characterisation, assessment, and classification of wild sericigenous insects.

Based on body marking and colour, six potential eri silkworm strains—YP, YS, YZ, GBP, GBS, and GBZ—have been identified from the Borduar and Titabar ecoraces. Two combinations, namely. Based on rearing results, YZ x YS and GBS x GBZ are deemed to be promising crosses. There has been one successful experimental grainage of these mixtures. In four states, namely Assam, Meghalaya, Arunachal Pradesh, and BTC, the NERTPS initiative is being used to conserve Muga and other wild silk moth species in-situ. Electroantennogram (EAG) was used to analyse the antennal responses of yellow fly throughout the feeding and spinning phases to volatiles isolated from the tasar host plant and Antheraea mylitta. Developed a natural module to combat muga silkworm illnesses and pests.

Post-cocoon R&D:

To manufacture better quality import-substitute silk, the development and demonstration of an indigenous automatic silk reeling machine (ARM). A demonstration of a solar-powered, reasonably priced spinning machine that may be used in rural settings. To boil Tasar cocoons more effectively, the chemical formula known as "Tasar Plus" has been devised. Technologies created to dry Tasar cocoons with hot air utilising a conveyor hot air dryer.

The widespread use of inexpensive eight-end, multi-end reeling machines for tasar silk reeling. Wet reeling of Tasar and Muga cocoons, Sizing Machine for Tasar Silk, Modified Dry Reeling Machine for Tasar Cocoons, Pressurised Hank Degumming Machine and Equipment for Recycling of Silk Reeling Water are all becoming more and more common in the field in the Vanya Silk Post Cocoon Sector. Created a conveyor cocoon dryer with a 1.2 MT/day cocoon drying capability.

Created the "Sonalika" reeling machine to replace the "Bhir reeling" of Muga cocoons. A demonstration of a machine that extracts the pellade layer from wasted silkworm pupae and separates the pupae. A technique known as "Use of Slug Catcher (as Replacement for Porcelain Button) for Slug Removing" has been developed and is now being field tested. Technology for "Yarn degumming using CSTRI Eco degumming machine" has been developed and is now being field tested. The Institute's vertical reeling machine has been improved and transformed into a three-ends machine for increased output. Developed fibroin matrix-reinforced Mulberry, Tasar, Muga, and Eri silk fabrics.

Technology that uses the HTHP approach to extract sericin from silk yarn has been developed. Created several Chanderi saree variants (Silk x Silk). Created Mulberry single jersey shirts for women with digital prints. Mulberry interlock knits with tie-dye yarn, Mulberry and cotton union knits with dye variation as a design element, and Mulberry jacquard tuck knit patterns for women's shirts were developed. Eri silk nonwoven textiles have been successfully manufactured, and studies on impregnation with cosmetic formulations for face mask application are now underway at L'Oréal. Developed Knits composed of Mulberry & Cotton Mélange Yarns. Describing the sericin's use in cosmetics (soaps, shampoos, hair treatments, etc.) and talcum powder applications as addictive. Technologies for cooking Raily Tasar cocoons for wet reeling have been developed. A biofinish has been created that considerably improves the visual and thermo-physiological comfort characteristics of Tasar materials. Developed technique to create a variety of outfits and knits utilising Indian silk of a high international standard. The Renditta has improved from 8.2 in 2005-2006 to 7.3 in 2016-2017 thanks to R&D work.

Commercialization & Patents:

1. Chemical formula for Muga cocoon heating for increased silk output

2. A handloom with improved lifting capabilities for jacquards uses pneumatics

3. Enhanced reeling and cum twisting equipment

Patent applications submitted:

1. Filed a patent application for the 'Rot Fix' product, an environmentally friendly composition with a wide range for treating toot rot in mulberries.

Commercialized technologies/products:

1. Products that have been commercialized include ANKUR, an organic and inorganic nutrient supplement for soil fertility and health, ANKUSH, a chemical formulation that is safe for the environment and is used to disinfect silkworm bodies and rearing seats, and Rot Fix, a formulation that is safe for the environment and is used to control the Root Rot disease in Mulberries.

For the purpose of extracting and characterising recombinant proteins, the full length lipoprotein gene was created and cloned in Pichia pastoris. To track the prevalence of illness in South Indian seed and commercial raising regions, surveys were carried out every two weeks.

From Bulgarian and Indian parents, two new breeding lines—the oval and dumbbell lines—were produced through the F3 generation. Six hybrids out of the 32 hybrids assessed in the artificial inoculation trial for root rot and root knot infections shown resistance to both diseases. Based on the results, two hybrid crosses with high production and better silk quality were found to be ICB14 x N23 and ICB17 x S8. Five pure breeds and two hybrids were purchased from Bulgaria in order to establish high producing silkworm breeds; they are now being assessed for further selection and use.

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CONCLUSION

With the use of comparative genomics, the pathobiome connected to the muga silkworm flacherie illness has been identified. The 68 to 72 hour old embryo represents the longest

period of embryonic development, which aids in creating appropriate schedules for egg preservation. Pebrine visualisation solution field tests for light microscopy identification of pebrine spores were carried out at several BSMTCs and PPCs in Jharkhand, Odisha, Chhattisgarh, and Madhya Pradesh. Pathogens linked to viral and bacterial infections that cause flacherie illness in Antheraea mylitta D. were located and isolated. Complete genome identification of the baulovirus that causes tiger band disease in Antheraea proylei (accession:GI 1371952746) For the purpose of preventing Bacillus sp.-caused bacterial flacherie illness, a novel chemical disinfectant has been developed. and Pseudomonas sp., which are present in the muga ecosystem and are the subject of ongoing bioassay research in a lab setting. At JSDI Ranchi, PPC Kharsawa, PPC Hatgamaria, and PPC Bengabad (JH), RTRS Baripada (OD), RTRS Bhandara (MH), REC Kapista (WB), and REC Kathghora (CH), a trial of the semi-synthetic food "Tasar Amrit" was conducted. Sericin has been isolated and characterised from waste tasar silk fibre in preparation for commercial use. About 1.8 to 2.5% of various fibre wastes contain sericin.

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CHAPTER 5
EXPLORATION OF SILKWORM HYBRID

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ABSTRACT:
Silkworms (Bombyx mori) have been essential to the manufacturing of silk, which has been a vital business for generations. This essay discusses the intriguing process of making silk and the prospect of developing a hybrid silkworm by fusing the DNA of Bombyx mori with those of other creatures, such as spiders. The historical context of silk production, the biology of silkworms, genetic engineering methods, prospective difficulties, ethical issues, and future ramifications are all covered in-depth in this paper. We hope to provide a thorough knowledge of the idea of generating silkworm hybrids and the numerous elements related to it in around 4000 words. For millennia, silk, known as the "Queen of Textiles," has been a representation of riches, refinement, and luxury. The silkworm, Bombyx mori, is at the centre of this enterprise, and its production has long been an integral part of human culture and commerce. A domesticated moth species called Bombyx mori has been deliberately cultivated for millennia to provide silk. In this essay, we examine the biology and history of silkworms, investigate genetic engineering methods that could produce hybrid silkworms, analyse the ethical issues involved in such research, and speculate on what the results might mean for the future of silk manufacturing

KEYWORDS:
Castor,Genotypes, Plants,Silkworm

INTRODUCTION

Silk has been made since ancient China, nearly 5,000 years ago. According to legend, silk was unintentionally discovered when a silkworm cocoon dropped into a hot cup of tea, unravelling the threads. Silk was a significant item in commerce along the Silk Road because the Chinese for generations kept the method of its manufacturing a secret. Ancient economies were significantly impacted by the silk trade, which also promoted contacts between East and West in terms of culture[1], [2].

The Silk Industry

The rearing of silkworms, especially Bombyx mori, is necessary for the manufacturing of silk. These tamed insects go through a metamorphosis process, spin silk cocoons, and only consume the leaves of the mulberry tree (Morus). The protein sericin, which binds the silk fibres together, is softened by boiling the collected cocoons, making it simpler to unravel the silk threads. After then, the raw silk is transformed, coloured, and weaved into textiles[3], [4].

Sericulture's Ascent

Sericulture, the practise of raising silkworms and producing silk, progressively extended from China to other Asian nations including Japan, Korea, and India. Eventually, methods for making silk made their way to Europe and the Mediterranean area, turning silk become a commodity on a worldwide scale. Due to the high demand for silk, silk farms were established and sericulture techniques were improved[5], [6].

The Life Cycle

The life cycle of Bombyx mori has four stages: egg, larva, pupa, and adult. The principal stage of silk production is the larval stage, often known as the silkworm. During this stage, silkworms consume mulberry leaves at a rapid rate in order to develop and save energy for pupation. They enter the pupal stage after they reach a certain size at which point they wrap themselves in a cocoon formed of silk strands[7], [8].

The Making of Silk

Silkworms are well known for their capacity to produce silk. By secreting a liquid from specialised glands that hardens when exposed to air, they make silk. The fundamental component of liquid silk is fibroin, a protein known for its extraordinary tensile strength. The figure-eight motion of the silkworm's head creates the distinctive cocoon structure as it secretes silk.

Genetic traits

Any efforts at hybridization must take into account Bombyx mori's genetic composition. Silkworms are diploid (two sets of chromosomes) creatures. They have around 31,000 protein-coding genes in their genome, which are used to make the different proteins involved in metabolism, digestion, and the synthesis of silk.

Concepts of Hybridization

In order to impart new features or qualities on Bombyx mori, the notion of producing hybrid silkworms includes adding genes from other animals. Enhancing silk quality or production effectiveness is the main objective in the context of silk production. Gene insertion, gene editing, and transgenesis are a few examples of genetic engineering methods that may be used to create hybrids.

Gene Insertion

The insertion of certain genes into the silkworm genome is known as gene insertion. Using this method, the quality and strength of the silk may be increased or genes that produce distinctive silk proteins can be added. To build hybrid silkworms that can produce spider silk proteins, scientists have experimented with DNA from other silk-producing creatures, such as spiders.

Gene editing

CRISPR-Cas9, a gene-editing technology that has recently made strides, has created new opportunities for modifying the silkworm genome. Existing genes, including those involved in the synthesis of silk, may be precisely modified via the use of gene editing. This strategy could result in the development of silkworms with enhanced silk qualities or disease resistance.

DISCUSSION

Genes are transferred from one species to another during transgenesis. Researchers have looked at the idea of developing transgenic silkworms that manufacture silk proteins from other species, such spiders or goats, in the context of the manufacturing of silk. These genetically altered silkworms may create silk with special qualities.

Potential Obstacles in the Development of Silk Worm Hybrids Technical Obstacles

The process of genetically modifying silkworms has a number of technological difficulties. It may be challenging to ensure that foreign genes are successfully incorporated into the genome of silkworms and to achieve sustained expression of these genes in glands that produce silk. The possible effects of genetic alterations on the silkworms' general well-being and viability must also be taken into account by researchers.

Considerations for Ethics

Concern has been raised about the moral ramifications of developing silkworm hybrids. Concerns concerning animal welfare are raised by genetic modification since scientists need to make sure the modified silkworms don't experience any negative consequences. Concerns about the discharge of genetically modified organisms into the environment and possible ecological effects also need to be addressed.

Economic and market factors

The introduction of hybrid silkworms might have an impact on the market and the economy of the silk producing sector. While the ability to produce silk with better qualities would enhance demand, traditional silk producers who are leery of genetically modified organisms might be resistant. The success of silkworm hybrids will largely depend on market acceptability and customer preferences.

Considerations of Ethics

The wellbeing of the organisms involved is one of the main ethical factors in the development of hybrid silkworms. The silkworms may suffer unforeseen consequences from genetic engineering approaches, such as aberrant development or decreased fitness. The welfare of these species must be given first priority by researchers, and they must make sure that genetic alterations do not cause needless suffering.

Environmental Effects

Environmental effects from releasing genetically altered silkworms into the environment are a worry. Analysing the possible hazards connected to the transfer of changed genes and their effects on regional ecosystems is crucial. To avoid unforeseen environmental effects, strong containment measures could be required. Silk manufacturing is the technique of using silkworms to generate natural silk fibre. Silk production is suitable for many production methods and may be carried out in a variety of agro-climatic settings. Silk manufacturing is a multifaceted practise that includes individuals from all walks of life in everything from host plant farming to silk processing. Additionally, the by-products have a variety of applications, from pharmaceutical businesses to fertilisers in rural regions, which might be accessed to eventually boost the income of farmers and other socioeconomic groups. Silk manufacturing has the potential to significantly boost the economies of several nations where there is an excess of labour, cheap production costs, and an openness to embracing new technology [9], [10].

Since the dawn of Ethiopian civilisation, silk has had a special place in the hearts of its people. However, the nation's silk threads were brought in from China, India, and Arabia [5]. Ethiopia is now the second-most populated nation in Africa. The unemployment rate is generally rising. Sericulture, an environmentally benign and labor-intensive cottage business based on agriculture, may therefore become a productive and efficient means of earning

revenue. In order to diversify exportable goods or import substitutes, slow the movement of people from rural to urban regions, and integrate byproducts into planting fields and the feed for fish and poultry, it is crucial to introduce and develop technology in Ethiopia [6–8]. As a consequence, several regions of the nation practise the manufacture of silk from Eri silkworms, particularly by impoverished farmers as a secondary source of income via the effective utilisation of family labour.Sericulture, also known as the breeding of silkworms, has been the subject of several research throughout history and is still being done today. Researchers have been working to develop a specific breed of silkworm that is low-cost to produce cocoons, adaptable to various agro-climatic conditions, disease- and pest-resistant, polyphagous, and produces high-quality silk. Therefore, by continual breeding, superior and resilient breeds have been created, which has shown a very large diversity in yield, quality, management, and demand for the environment.

The main source of food for the Eri-silkworm (Samia cynthia ricini Boisduval) (Lepidoptera: Saturniidae) is castor (Ricinus communis). The castor (R. communis), Heteropanax fragrans, Evodia flaxinifolia, and Manihot utilissima are only a few of the plant species that the Eri silkworm feeds on [11, 12]. But the primary host plant for the Eri-silkworm is castor. Eri-silkworms raised on castor leaves produce huge, silk-rich cocoons. The development of the silkworm and, eventually, the economic characteristic of cocoons are greatly influenced by the quality of the diet. Castor leaves are primarily used in the raising of Eri-silkworms because they offer the greatest results in terms of the qualitative and quantitative properties of the Eri-silk.

One of the most exploited, tamed, and commercially successful nonmulberry silkworms is the eri-silkworm. It reproduces often each year and feeds on a variety of host plant species. It can be cultivated inside since it is a tamed silkworm. R. communisis is the Eri-silkworm's favourite host plant out of all the others. Additionally, without impacting the production of oilseeds, roughly 25–40% of castor foliage may be defoliated (removed) and utilised as food for Eri-silkworms. As a consequence, one ongoing activity in the realm of silk production is the breeding of silkworms. A key element impacting the creation of a high-quality cocoon has been identified as the quality of the leaves fed to the Eri-silkworm. The genotype of the castor plant and the calibre of the leaves supplied to the worms have been shown to have an impact on growth, development, and cocoon output. In order to raise silkworms, it is crucial to have a high-yielding and high-quality genotype of castor. The purpose of this research was to assess the effects of castor genotypes and their feeding characteristics on Eri-silkworm rearing efficiency.

The Study Area's Description

The experiment was carried out at Tepi Agricultural Research Centre, which is 610 kilometres from Addis Ababa, the nation's capital city, in southwest Ethiopia. It is 1200 metres above sea level. With a minimum annual rainfall of 600 mm and a maximum annual rainfall of 1500 mm, and with a minimum and maximum annual temperature range of 20 to 28°C, respectively. The region has a high humidity level and a diverse range of plants and animals.

Experimental Materials and Design

A total of 34 castor genotypes were planted at the research centre for screening reasons after being collected from various regions of the nation. Then, ten castor genotypes were chosen from a total of thirty-four genotypes for this experiment depending on how well they

produced yields. Additionally, Eri-silkworm eggs from the Melkassa Agricultural Research Centre were transported in order to assess how well the Eri-silkworm performed while feeding on various castor genotypes. Ten castor genotypes' agronomic performance was assessed using a randomised full block design with three replications of the treatments. To find the optimum castor genotypes to feed Eri-silkworm, a feeding experiment was carried out in the lab. Three replications of each treatment were used in the entirely randomised design of the treatments. 100 worms were utilised in each replication, and they were given time to complete the larval stage.

Experimental Techniques

The castor genotypes were planted with a distance of 75 cm between rows and 70 cm between plants. Each plot had four lines and 16 plants, and the size of the plots was maintained at 2.8 3 m. The two centre plant rows in each plot were considered the net plot, while the two outside plant rows were designated as border rows. As a sampling point for data measures, four sample plants from net plots were collected. The distance between blocks and plots was two metres and one metre, respectively. Except for the variations in their genetic make-up, the castor genotypes were planted and tended in each plot in a comparable manner. The first weeding was carried out 30 days after seeding, and the subsequent weedings were carried out 60 and 90 days later, respectively.

Before the experiment (raising) began, the silkworm rearing space and its associated equipment were cleaned, rinsed, and disinfected using a 2% formalin solution at a rate of 800 ml per 10 m2. For this experiment, the mixed strain of Eri-silkworm was employed. Young age instars (1st–3rd) were given freshly harvested shoots that had been sliced to a size suitable for the developing larvae. The fourth and fifth age instars, however, were fed medium to mature leaves. Young larvae were given delicate, succulent, and nutrient-rich leaves, which are known to promote the growth and development of silkworms. When the larvae reached ripening, they were fed mature and coarse leaves. The normal daily feeding schedule for each race of silkworms was four times daily. Every morning before the first feeding, the rearing beds were cleaned. According to guidelines, the room's temperature and relative humidity were maintained. Ages of the mount were planned to coincide with adult worms. After mounting for seven days, the cocoon produce was gathered.

Data Collected

The three-month plant height, 50% emergency date, leaves per plant, number of primary branches, 50% flowering date, internode length, ten fresh leaf weights (g), ten dry leaf weights (g), leaf area (cm2), seed yield/plant (g), disease and pest incidence, and number of racemes per plant were the agronomic data of the castor genotypes that were gathered. The number of larvae remaining after each moulting stage under observation (at 1st–4th instars), the total number of larvae reaching full maturity, the weight of ten matured larvae (g) at 5th instar at 6 days of age, the developmental period (egg, larvae, pupae, and adult longevity), the date of mounting, the date of harvesting, the fresh weight of single cocoons (g), and the fresh wei weight were all collected during the study period.

Effect of Different Castor Genotypes on Eri-Silkworm Rearing Performance

The 200390 (92.68%) and 106564 (86.00%) genotypes given to silkworms resulted in the greatest and lowest hatching percentages, respectively. All treatments, however, showed no evidence of a meaningful change (Table 3). This result supports the findings of Sarkar et al, who observed that feeding on several castor genotypes increased silkworm hatchability from

90% to 85%. Additionally, Sannappa et al. [26] found that feeding on various castor genotypes resulted in hatchability varying from 98.05% to 98.92%. In a separate research, Shifa et al. [8] found that the egg hatchability of Eri-silkworm larvae fed on several genotypes of castor varied significantly, from 81.50% to 95.33%.

The variance observed in the Eri-silkworms fed on various genotypes of castor may be related to changes in the foliar component composition of the genotypes of castor that led to differences in larval duration. Similar to this, a substantial difference in Eri-silkworm larvae weight fed on castor genotypes was observed (). Significantly, Eri-silkworms fed the Abaro genotype had the greatest larval weight (6.16 g).

Significantly , the Wolenchite genotype had the lowest cocoon weight (3.08 g) while the Eri-silkworm genotype had the greatest (3.26 g). The silk ratio showed a significant variation, with a greater value (13.80%) obtained for the larvae fed on the 219645 genotypes (Table 4). This result is consistent with those of Pandey [34], Ahmed [35], and Sarkar et al. [25], who found that various castor genotypes supplied as feed caused variations in cocoon characteristics. In the current research, Eri-silkworms fed with the 219645 genotypes with considerable diversity had the maximum shell weight (0.45 g) and pupal weight (2.46 g). However, Eri-silkworm fed on the Wolenchite genotype had the lowest shell weight (0.40 g) and pupal weight (2.12 g). distinct genotypes of castor leaves may have distinct chemical compositions, which might account for the diversity in shell, pupae, and cocoon quality [11], [12].

CONCLUSION

Castor genotypes and environmental differences may have a role in this variance in hatchability. The Eri-silkworm hatchability is directly correlated with the foliar components of the castor genotype. When Eri-silkworm consumed several castor genotypes, a discernible difference was seen in the effective rate of rearing (ERR). According to Table 3, Eri-silkworm fed on genotype 200390 (94.54%) had the greatest ERR, followed by genotypes Abaro (90.60%) and 219645 (88.32%). The changes in foliar composition and nutrient availability in various genotypes that affect the growth and development of silkworms may be the cause of the variance in ERR of silkworms fed on different castor genotypes. Observing changes in ERR as a result of variances in castor genotypes, Chandrashekhar and Govindal , and Gurajala and Manjula [28] reported a similar discovery. The current research also shows that Eri-silkworm larvae fed on various castor genotypes had significantly varying larval durations. The larval duration of eri-silkworm fed on the 200390 and 106564 genotypes was shorter. A substantial difference was also seen in the length of the whole life cycle of Eri-silkworm larvae fed on various genotypes, with the 200390 genotypes having the shortest life cycle at 58 days. Several scientists made a similar discovery, reporting that various castor genotypes resulted in varying silkworm larval lengths.

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CHAPTER 6
ANALYSIS OF SILKWORM BIOTRANSFORMATION FROM MULBERRY LEAVES AND SILKWORM DROPPINGS

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ABSTRACT:
Silkworm intestines, a valuable source of medication in traditional Chinese medicine , produce silkworm droppings when they ingest mulberry leaves. By using HPLC, GC-MS, and UHPLC-Q-TOF MS, the total fat, fat acids, crude protein, amino acids, and secondary metabolites of the collected mulberry leaves and silkworm droppings were examined. Network pharmacology was used to examine the target genes and enriched pathways connected to the drastically altered compositions between mulberry leaves and silkworm droppings. Mulberry leaves and silkworm excrement both converted high unsaturated C18: 3 fatty acids to low unsaturated C18: 1. Only 17 mini-peptides and lysine were substantially more abundant in mulberry leaves than in silkworm droppings. Between mulberry leaves and silkworm droppings, there identified 36 similar target genes or distinct chemical components. While the primary pathways of silkworm droppings were concentrated in hormone regulation and signal transduction, those of mulberry leaves were enriched in anti-virus and anti-cancer capabilities.

KEYWORDS:
Amino Acids, Droppings, Leaves, Mulberry, Silkworm**.**

INTRODUCTION

In China and other Asian nations, traditional Chinese medicine has a long history and has been used to prevent and treat a wide range of illnesses. Traditional Chinese medicine uses mulberry leaves, silkworm droppings, and other substances as therapeutic agents. ML are essential to the sericulture sector since silkworms only eat them. About 60% of the mulberry leaves that the silkworm ingested were ejected without being digested, resulting in droppings , which are made up of mulberry leaf material as well as different substances that were changed by enzymes and microorganisms in the caterpillar's gut . Silkworm droppings are used in traditional Chinese medicine to expel wind, harmonies the stomach, transform turbidity, disperse dampness, activate blood, and encourage menstruation. ML is used to dispel wind-heat, moisten the lungs, soothe the liver, and brighten the eyes. The metabolism of silkworms may be the root of their varying efficacies. They shifted from having cool to warm cold/hot natures. One of the fundamental and guiding ideas in traditional Chinese medicine and clinical treatment is the cold/hot natures hypothesis of Chinese materia medica. This process involves biotransformation, which includes fermentation and the biotransformation of tissues and organs [1], [2].

Widely found in tropical, subtropical, and temperate regions, Morus alba L. is a fantastic source of nutrients and phytochemicals . Organic acids and macro- and micronutrients may be found in abundance in ML. Fatty acids, amino acids, polysaccharides, alkaloids, volatile oils, and other active substances with potent antioxidant, antibacterial, anti-inflammatory, hypoglycemic, and lipid-lowering properties are found in ML. The amount of -aminobutyric acid in ML tea is ten times more than that in green tea. Chlorophyll and its derivatives,

xanthophyll, carotenoid, flavonoids, and other chemicals are the main chemical components of silkworm droppings. Additionally, the lipid compositions in SD included high amounts of liposoluble substances as phytosterol, unsaturated fatty acids, and fatty alcohol. The ML and SD have several targets, components, and pathways. However, there is still a lack of knowledge on the pharmacology and mechanisms of several drugs[3], [4].

Chemical metabolites, network pharmacology, and bioinformation are only a few of the recent methods that have been investigated in order to characterise the properties of Chinese medicinal ingredients. For the purpose of assessing the quality of natural products, the HPLC, GC-MS, and UHPLC-Q-TOF MS are crucial methods. In this study, differential metabolomics and network pharmacology were used to examine the difference pharmacology and composition of ML and SD. Different long-chain fatty acids and amino acids all have considerably different compositions. The ML and SD of 386 compounds were found to vary. Virus infection and cancer signalling pathways were primarily enriched in the target genes of high content chemicals in ML. The neuroactive ligand-receptor interaction, bile secretion, insulin resistance, and signalling pathway were the three areas where high content chemicals in SD were most concentrated in target genes. New information on the pharmacology and pharmacodynamics of ML and SD may be gained from the findings[5], [6].

Sample Preparation for a Metabolomics Study

From a mulberry garden at Zhejiang University in Hangzhou, Zhejiang Province, China, mulberry leaves were collected. Each leaf was split into two pieces symmetrically. In an electric grinder, the other half of the leaves were crushed to a fine powder after being dried at 40°C. To get the intraday SD, the remaining half of the leaves were given to the Qiufeng Baiyu fifth instar third-day silkworm. After being collected, the silkworm droppings were dried at 40°C and powdered in an electric grinder. The Zhejiang Academy of Traditional Chinese Medicine's biobank included the powdered mulberry leaves and silkworm droppings.

Determination of Crude Fat and Fatty Acid Content

The filter paper tube-wrapped 1.0 g sample was then placed into the Soxhlet extractor. Oil ether was added, and the mixture was extracted in a water bath at 40 °C for 6 to 8 hours. Recycling extract and filter paper tubes were dried and weighed to determine the total fat content after the extraction. 1 g of the sample was placed in 2 mL of petroleum ether, 2 mL of n-hexane, and 2 mL of a 0.4 m KOH/CH3OH solution to soak for the night. The top extract was dried and redissolved with n-hexane after the saturated salt solution was added and stratified. By using GC-MS, the 1 l of fatty acids extract was examined[7], [8].

Determination of Crude Protein and Amino Acid Content

The digester was filled with the 0.5 g sample, 0.4 g CuSO4, 6 g K2SO4, and 20 mL H2SO4 and digested at 420°C for one hour. After the sample had cooled, 50 mL of water was added in order to carry out the semiautomatic Kjeldahl nitrogen metre titration and determine the crude protein level. The 0.5 g sample was hydrolyzed with 10 ml 6 N HCl at 110 °C for 22 hours. After drying, 2 mL of 0.1 N HCl was used to re-dissolve the top extract. Liquid chromatography was used to determine the amino acid content.

DISCUSSION

A 100 mg aliquot of the material was extracted with 1000 L of a 3:1 methanol to water solution on a shaker overnight at room temperature before being centrifuged at 12500 rpm for 15 minutes. A 100 x 2.1 mm, 1.8 m Waters ACQUITY UPLC HSS T3 column was used for

the UHPLC separation. Mobile phase B comprised acetonitrile, while mobile phase a contained 0.1% formic acid in water. The temperature in the column was fixed at 40 C. The injection volume was 2 L, and the autosampler's temperature was set at 4°C. In an LC/MS experiment, the QE Focus mass spectrometer was used because of its capacity to collect MS/MS spectra on an information-dependent basis. In this mode, the MS/MS spectra collection is triggered based on preset criteria, and the acquisition software continually examines the full-scan survey MS data as it is collected. Three precursor ions with intensities larger than 5000 were selected for collision energy fragmentation in each cycle. With three injections, the acquired mass range was split into three ranges: 70–300, 290–600, and 590–1100. Spray voltage was adjusted at +3500/-3500 V, capillary temperature to 350 °C, and sheath gas to 30, aux gas to 10, and CE to 10, 30, and 50. AB For the development of the test, a Sciex QTrap 6500 mass spectrometer was used. Ion spray voltage: +5500/4500 V, curtain gas: 35 psi, temperature: 550°C, ion source gas 1: 60 psi, ion source gas 2: 55 psi, and DP: 100V were typical ion source characteristics.

Preprocessing, Annotation, and Compound Analysis of UHPLC-MS Data

ProteoWizard was used to convert the high-resolution MS data to the mzXML format, and MAPS software was used to analyse it. The peak intensity, mass-to-charge ratio, and retention time measurements were all produced as a consequence of the preprocessing. Metabolites were identified using an internal MS2 database, and Skyline software was used to analyse MRM data. The resulting datasets were identified and aligned, and then SIMCA-P software was used to undertake multivariate statistical analysis, including supervised partial least squares discrimination analysis. The effectiveness of PLS-DA and orthogonal partial least squares discriminant analysis models was evaluated using parameters like R2 and Q2. By using the variable significance in the projection values, the distinguishing characteristics were retrieved.

Network Pharmacology Analysis

We searched databases using SymMap and Traditional Chinese Medicine Systems Pharmacology to find the bioactivity of various chemical components between μ ML and SD. Different chemical components' target genes were discovered. ClusterProfiler enhanced the GO function and KEGG pathway of the target genes. Cytoscape was used to establish the relationship between the component, target, and route.

Various analyses of the crude protein and amino acid contents

To attain the proper structure and function, the protein must be ingested as a component of a diet that is otherwise nutritionally sufficient. Amino acids are necessary for the body's protein synthesis as well as the production of hormones and neurotransmitters, which include nitrogen. According to Figures 2 and 2 and Table S2, the amount of crude proteins and amino acids had a significant impact on ML and SD. In comparison to SD, ML had larger concentrations of alanine, serine, asparagine, isoleucine, glycine, cysteine, threonine, phenylalanine, and glutamic acid and Table S2). However, SD had a greater lysine content.

The metabolic differences were characterised using OPLS-DA in order to make the metabolic alterations of ML and SD more understandable. By using the OPLS-DA permutation test, the samples from ML and SD = 0.816, R2Y = 1, and Q = 0.998) were clearly differentiated based on the differences in their whole metabolic profiles and S2). These findings showed that eating of silkworms significantly altered the metabolic profiles of ML and SD.

The VIP was created using OPLS-DA, and the value was determined using a Student's t-test to determine the contribution of the differentiated metabolites. Between ML and SD, the 386 distinct compounds were discovered and Table S4). In comparison to SD, ML had a greater concentration of 156 chemicals . Among all the distinct molecules, there were 83 mini-peptides. In ML, there were 66 more mini-peptides than in SD, whereas in SD, there were 17 more mini-peptides than in ML. Differentiated compounds' KEGG pathways were examined. The principal differential chemical synthesis routes were flavonoid biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis).

Network Pharmacology Analysis

The TCMSP and SymMap databases were searched for the 303 distinct compounds, excluding the 83 mini-peptides, and information on 32 components was found . While the contents of 19 compounds were greater in SD than ML, the contents of 13 components were higher in ML. Also looked for were 32 components in ML and SD's linked target genes. Using Cytoscape software, the compound-target-pathway network was built depending on the magnitude of a topological parameter.

Commonly used in traditional Chinese medicine are ML and SD. Due to the digestion of the silkworm, the medicinal actions of ML and SD are comparable yet distinct. Thin-layer chromatography patterns revealed differences in more than 50% of the components between MeOH extracted ML and SD. Organic acids, flavonoids, and alkaloids such gallic acid, fumaric acid, chlorogenic acid, quercetin, and 1-Deoxynojirimycin are among the active components in ML . Alpha-glycosidase, which is involved in the breakdown of carbs, is inhibited by 1-DNJ, which stops sugar from entering the circulation. Additionally, it has been noted that the ML extract inhibits the absorption of cholesterol in the gut, which has been linked to a reduction in hyperlipidemia and atherosclerosis. Antioxidant capabilities are seen in the polysaccharides of ML. Mulberry leaf extract is resistant to methotrexate-induced hepatotoxicity. Chlorophyll, vitamins, and metal complexes of porphyrins may all be found at little cost in SD. IDA has successfully employed SD extract as an oral iron supplement. By controlling the Th1/Th2 immune response, SD extract reduces a variety of allergy symptoms.

Lipids, which comprise fatty acids and cholesterol, are crucial nutrients for health. In the meanwhile, cavitating oil-water fluxes and oil viscosity have an impact on the effectiveness of lipid extraction in various containers. In our study, petroleum ether was used to extract the materials in a Soxhlet extractor. Lipid molecules are crucial membrane building blocks and signalling pathway mediators. Through oxidative and inflammatory stress, saturated and unsaturated fatty acids may influence the progression of cardiovascular disease . Although there was no discernible change in the amount of crude fat between ML and SD, there was a discernible variation in the amounts of several long-chain fatty acids . In nonalcoholic fatty liver disease , the lack of C15:0 fatty acids causes liver damage . Oleic acid is a metabolite of fatty acids that might impact embryo development . Oleic acid has anti-inflammatory properties and is used as a substitute to treat inflammatory skin conditions . The linolenic acids were effective inhibitors of both glycation and advanced glycation end products . Linolenic acid reduces acetylcholine-induced relaxation by preventing the synthesis of cGMP, which is brought on by nitric oxide . In addition, lipid may be used as an oral drug delivery strategy to ensure the drug's solubility and prevent vascular embolisation .

Proteins, which are composed of amino acids, carry out almost all of the tasks necessary for cellular life, such as acting as pumps or catalysts. The human immune system, particularly the T-cell system, is compromised by the protein diet . Compared to other green leafy

vegetables, ML has a much greater protein content . According to our research, ML had a considerably greater crude protein and amino acid content than SD . In comparison to ML, SD had a greater concentration of lysine). This is a result of proteins and amino acids being digested and absorbed by the digestive system of silkworms. Lysine may trigger humoral and cell-mediated inflammatory, immunological, and angiogenic responses to speed up the healing of all kinds of wounds. The plasma amino acid content rises as a result of abnormalities in amino acid metabolism . Despite making up just 2% of the protein, cysteine is the most common posttranslational modification . Despite being a non-essential amino acid, cysteine deficiency has been linked to neurodegenerative disorders[9], [10] .

The Chinese medicine medica may be fully characterised by chemical metabolomics and network pharmacology, which reflect various components and multiple targets . The "holistic" viewpoint of TCM and the impact of Chinese materia medica are congruent with the thorough investigation of metabolomics and network pharmacology. To find possible indicators for the special therapeutic capabilities of mulberry leaves and silkworm droppings, the distinct components and metabolic pathways were examined. In contrast to silkworm droppings, mulberry leaves had larger concentrations of kaempferol and quercetin, which are necessary for the formation of flavone and flavonols and Table S4). The anti-cancer, anti-inflammatory, and antiviral properties of flavonoids in ML are advantageous . In comparison to mulberry leaves, silkworm droppings had higher concentrations of benzene and its substituted compounds, carboxylic acids and their derivatives, fatty acyls, and prenol lipids . These findings imply that the intestinal metabolism of silkworms enhances the complexity of the chemicals found in mulberry leaves. However, several chemicals in silkworm sand have targets that are similar to those in mulberry leaves). The primary target pathways of differing chemicals in mulberry leaves compared to silkworm droppings are viral infection and cancer signalling pathways . These many biochemical connections between mulberry leaves and silkworm excrement may be responsible for their various therapeutic effects[11], [12].

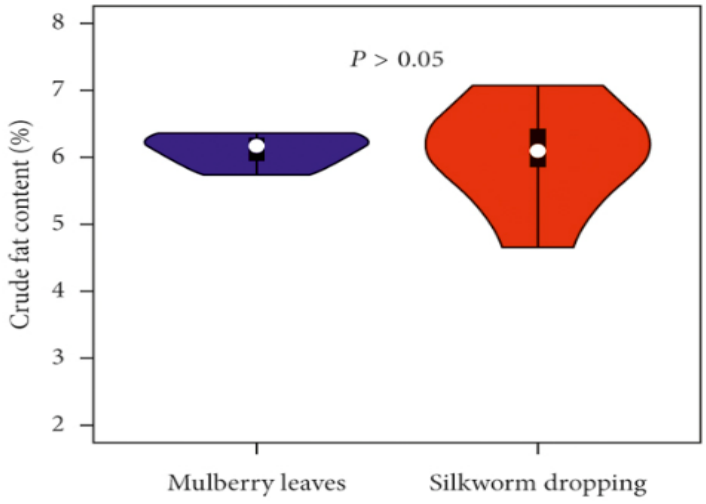

Figure. 1 shows the crude fat content percentage in mulberry leaves and silkworm dropping

CONCLUSION

Metabolomics and network pharmacology were used to analyse the pharmacodynamic substance bases and the pharmacological targets of ML and SD. After being digested by

silkworm intestines, the fatty acids, amino acids, and flavonoids in SD were dramatically altered in comparison to those in ML. Cancer and antiviral are prominent in the main mulberry leaf route, while signal transduction and hormone regulation are abundant in the major SD pathway. These findings may be connected to ML and SD's traditional Chinese therapeutic characteristics, and they indicated that intestinal digestion and silkworm absorption were key factors in the alteration of ML and SD's pharma-codynamic substance base and pharma-codynamic activity. This investigation would provide fresh information on the biotransformation of Chinese medicine.

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CHAPTER 7
SILKWORM SERICIN: PROPERTIES AND BIOMEDICAL APPLICATIONS

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ABSTRACT:
Bombyx mori silkworms naturally manufacture silk sericin, a polymer that encases and holds two fibroin filaments in the silk thread needed to make cocoons together. Sericin is often dumped by the textile industry, and its recovery and reuse not only reduces environmental problems but also has significant scientific and economic significance. The extraction process and silkworm lineage have an impact on the molecule's physicochemical characteristics, which may cause differences in sericin's molecular weight and amino acid content. These variations have several uses in biomedicine. The ability to act as an antioxidant and the presence of very hydrophobic amino acids allow sericin to be used in the food and cosmetic industries. The moisturising ability enables use as a medicinal agent for wound healing, promoting cell proliferation, UV protection, and cream and shampoo formulation. Sericin's low digestibility and antioxidant activity increase its medical applications as an antitumor, antimicrobial, anti-inflammatory, and anticoagulant, as well as their effects on colon health, constipation relief, and the prevention of obesity through improved plasma lipid profiles. Additionally, sericin's characteristics enable its use as a culture medium, for cryopreservation, in tissue engineering, and for drug administration, illustrating its usefulness as a crucial biomaterial.

KEYWORDS:
Cocoon, Fibroin, Protein, Sericin, Silk.

INTRODUCTION

The silkworm, Bombyx mori, a holometabolous insect of the Lepidoptera order and Bombycidae family, produces the protein sericin. B. To the conclusion of the fifth larval instar, the mori produces a significant quantity of sericin, which, together with fibroin, is utilised to make the silk thread that is needed to create the cocoon, a structure that creates the optimal circumstances for the occurrence of larval metamorphosis to adults .In the textile business, a procedure known as degumming is used to treat the cocoon and mostly eliminate sericin. The creation of several varieties of yarns and silk textiles uses the fibroin, which is transformed into raw silk . One of the first agricultural discoveries made by humanity is commercial silk growing, according to research . North China is the source of historical information that dates back 5000 years. where it has spread to more regions in the East and West . In addition to the economic value derived from agriculture uses, B. The primary lepidopteran employed in scientific study is mori, a genetic resource that may provide light on a variety of biological issues . The B.'s cocoon was recently. Research has indicated the potential utility of mori and its two major proteins, fibroin and sericin, in the fields of polymers, biomaterials, cosmetics, and the food sector[1], [2] .

Sericin has been ignored in the area of sericulture for a very long period. According to estimates, 400,000 tonnes of dried cocoons are produced globally each year, yielding 50,000 tonnes of sericin, most of which is dumped in wastewater . As a result, there is a significant

increase in the need for chemical and biological oxygen and water pollution . Particularly in nations where sericulture is practised, like China, India, and Brazil, the removal and usage of sericin might have a significant negative influence on the economy, society, and ecology. A natural polymer called sericin serves as an adhesive to unite two fibroin filaments to create silk yarn . With a molecular weight range of 20 to 400 kDa and 18 amino acids, including the essentials, the molecule is very hydrophilic. Sericin has unique properties as an antioxidant, moisturising, healing, antibacterial, antimicrobial protection against ultraviolet radiation, and antitumor agent thanks to the polar groups of amino acid side chains as well as its organic composition, solubility, and structural organisation . Thus, the growing interest in nontextile uses of silk protein in a wide range of scientific and medical fields is evident in the demand for biocompatible and biodegradable materials, which supports the current review of the properties and biological uses of sericin polymer derived from B. cocoons

Development of the Silk Gland and Sericin Synthesis

The labial gland of B is where sericin is made. Mori, also known as silk glands, are two tubular organs that stretch lateroventrally from the labial segment to the caudal section of the digestive canal. In B. At the conclusion of each instar, the rudimentary gland in mori secretes a little quantity of silk that is utilised to attach the tegument that will be shed during moulting to the substrate. The fifth instar sees the development of gland hypertrophy, which results in an increase in cell volume, high levels of silk production and secretion, and weight that ranges from 20% to 40% of the insect's overall weight .The anterior silk gland , which forms the excretory duct and has about 200 cells, the middle silk gland , which secretes three types of sericin and is about 7 cm in length and has about 300 cells, and the posterior silk gland , which secretes fibroin and is about 15 cm long and has about 500 secreting cells, are the three regions that make up the silk gland, a typical exocrine gland . Figure 1 shows a pair of silk glands, each with its own distinctive areas. The organ responsible for spinning silk, known as the spinnerets, is where the anterior area finishes. The anterior, anterior-middle, posterior-middle, and posterior regions make up the middle region. Each of these regions produces a unique form of sericin in the lumen, and these differences are caused by the density and shape of the material contained in secretory vesicles [3], [4].

The formation of the silk gland begins during embryonic development and continues throughout the larval stage, when endomitosis—a process in which cells replicate their DNA without going through mitosis—occurs .The simple glandular epithelium depends on a continuous basal lamina to divide the hemocoel and morphologically resembles the posterior and middle sections. The ramified nucleus of secretory cells, which contains a significant quantity of genomic DNA that has been replicated 200–400 times , is shown to have multiple nucleoli scattered throughout . The core surface is regular at the start of larval development but becomes complicated as it progresses. Ichimura et al. state that nuclear ramification is first seen near the end of the third instar. Rough endoplasmic reticulum , the Golgi complex, and mitochondria are only a few of the many organelles found in the cytoplasm that are involved in the production of secreted proteins . The posterior part of the silk gland secretes the inner layer of sericin, which instantly gathers around the fibroin due to the peculiarities of the central region of the silk gland. For the posterior-middle region, a layer with a more granular texture and lower density is placed on top of the inner layer. The prior field, which comprises the biggest cells distinguished by the presence of fat bodies, produces the outermost layer of sericin .

As a result, sericin organises into three layers surrounding the two fibroin filaments that emerge from each of the silk glands in the silk thread . Liu and Zhang provide an overview of the process by which soluble silk proteins create an insoluble silk thread. The fibroin migrates to the middle area where it is surrounded by sericin after being produced into the glandular lumen in solution form with around 15% protein[5], [6].

DISCUSSION

The glandular epithelial cells gradually take up water, creating a gel-like solution that contains 30% protein and has the property of nematic liquid crystal. Proteins are transported into the anterior silk gland duct when a spinner is in motion; here, more water and ions are absorbed, and the crystalline liquid solution eventually solidifies to become a solid filament. Additionally, a consistent and focused movement of B. As the silk proteins aggregate and crystallise, they become more hydrophobic and cause the loss of water on the thread's surface . Mori head during spinning also affects the orientation of protein molecules in the silk thread. The process of creating a cocoon takes approximately three days and starts from the outside in, when silk strands are "glued" together using the sericin's glue-like properties. A lengthy silk thread called a cocoon, which may vary in length from 900 to 1500 metres thanks to millions of years of evolution, offers the best defence from harmful environmental factors and biological threats including bird, insect, and bacterial assaults during the transformation into a silk moth. Besides the existence of the p25 protein and seroin, which are also released by the silk glands, and which make up 98% of the structure, its key proteins, fibroin and sericin, are also important for the resistance to predators, fungi, and bacteria. The cocoon also contains other materials such lipids and waxes , inorganic salts , and pigment . Silk glands go through morphological and functional changes as a consequence of insect metamorphosis, and they totally degenerate 48 hours after the pupal stage starts[7], [8] .

The structure and genetics of silk proteins

There are two different protein families called sericin and fibroin. A glycoprotein called fibroin fibre is released into the lumen of the posterior glands as a molecular complex made up of a heavy chain that weighs around 350 kDa, a light chain that weighs 25 kDa, and a P25 chain that weighs 27 kDa. On chromosomes 14 and 25, respectively, are the L and H genes placed . Microfibrils of fibroin are grouped into fibril bundles, which when combined create a single silk strand. The two filaments that make up the silk thread during cocooning are encased in layers of sericin, each of which originates from a silk gland.

Biochemistry of serin

Sericin is a member of a family of "gluelike" proteins that surround the protein core and hold the fibroin filaments together . Silk fibre is harsh and rigid when sericin is present, but soft and glossy when it is missing .Sericin is a globular protein with random coil and -sheets in its structure. The temperature at which the sol-gel transition takes place, mechanical stretching characteristics, and moisture absorption all readily cause changes in the random coil topology of the -sheet. Protein takes on its soluble form in water that is at least 50 to 60 °C hot. Lower temperatures cause the solubility to decrease and the random coil structure to change into -sheets, which causes a gel to develop .The 18 amino acids that make up a macromolecule with a hydrophilic character include strong polar groups including hydroxyl, carboxyl, and amino groups . These groups may create crosslinks, copolymerizations, and combinations with other polymers . 46.5% carbon, 31% oxygen, 16.5% nitrogen, and 6% hydrogen make up its organic makeup . Sericin has significant biological features due to its biochemical

makeup, including biocompatibility, antibacterial activity, antioxidant capacity, and moisturising effects, among others .

Types of serin

Sericin's solubility and molecular weight are characteristics that may serve as a benchmark for categorization. According to its solubility in water, Shaw and Smith divided the sericin into three parts . The outermost layer of the cocoon includes sericin A, which is more soluble in warm water and contains 17.2% nitrogen with serine, threonine, glycine, and aspartic acid as the main amino acids. Sericin B, which includes 16.8% nitrogen and an addition of tryptophan and is made up of the same amino acids as sericin A, is located in the intermediate layer. Sericin C, the last fraction, is located in the innermost layer next to fibroin; it is insoluble in hot water and has a reduced nitrogen content . Fraction C includes proline in addition to the amino acids present in sericins A and B. Takasu et al. categorised sericin as sericins A, M, and P, which contain the three major polypeptides that make up the protein, based on the location of synthesis in the middle part of the silk gland. The first and second sericin layers that include the fibroin are formed, respectively, by sericins P and M, which are encoded by the Ser1 gene . Up until day 6 of the 5th instar, MSG expresses your transcripts in the posterior and middle regions but not in the anterior region. The Ser2 gene is expressed in the anterior region; it is almost ever seen in the middle region and is not present in the posterior region. Your facial expression was visible up until day 4 of the sixth instar and then it vanished on day 6. Sericin A, which is typically found in the inner and outer layer of the cocoon and is generally found in the anterior section and seldom in the middle portion, is encoded by the Ser3 gene. Beginning with the fifth instar, the Ser3 transcript signal starts on day five and becomes stronger all the way up to day seven.

While the sericin layers in B are made up of the Ser1 and Ser3 gene products. The proteins expressed by the Ser2 gene are categorised as noncocoon and are connected to larval silk . Before each instar shift and before the cocoon is produced, a little quantity of silk is created by the silkworm, which anchors the cocoon to a suitable substrate .Following the proteolytic breakdown of sericin, Sinohara and Asano recovered glycopeptides that showed the presence of glucosamine, galactosamine, mannose, and galactose. Sinohara discovered that B. There are two different kinds of oligosaccharide units in mori sericin. One is made up of two N-acetylglucosamine residues and multiple mannose residues, one of which is connected to an asparagine residue in the protein core. The second oligosaccharide unit is made up of an isolated N-acetylgalactosamine or a disaccharide called -galactosyl -N-acetylgalactosamine, which is joined to the protein's amino acid core through a serine or threonine residue.

According to Kodama , the sericin molecule changes somewhat when heated at high temperatures , which are employed during the extraction in autoclaving. Additionally, the author demonstrated that sericin has an isoelectric point that is somewhat more acidic and is soluble in water. According to Aramwit et al. , degumming using heat or heat under pressure has the benefit of producing no impurities. When sericin is extracted from cocoons, the preparation circumstances, such as temperature, pressure, and heating time, may significantly influence the molecular weight of sericin. In particular, the circumstances of the extraction may influence the molecular weight of sericin .

Takasu et al. demonstrate that sericin is composed of three major polypeptides, with molecular weights of 400, 250, and 150 kDa estimated by SDS-PAGE, which correspond to sericins M, A, and P, respectively. They do this using a saturated aqueous lithium thiocyanate containing 2-mercaptoethanol solution with ethanol precipitation. Kurioka et al.'s study

compared the morphological and biochemical properties of the protein obtained with an alkali-degraded protein and high temperature and pressure . The yield of sericin powder is the same using all three extraction techniques. Morphologically, by looking at SEM pictures, thin films with a leaf-like structure may be seen in the acid degumming process, however they are less in size than the alkali- and heat-degumming processes. Trypsin inhibitor activity was reduced by 31% during heat extraction at 110°C, or 60% less than it was during acid extraction. Trypsin inhibition is unaffected by the alkali-degumming. When the temperature was raised to 115°C or 121°C, the trypsin inhibitor activity fell by 60% and 75%, respectively[9], [10].

The chemical composition and antityrosinase activity of sericin extracted using different techniques were studied by Aramwit et al. B. cocoons are used for extraction at high pressure and temperature. Mori were autoclaved for 60 minutes at 120°C and 15 lbf/in2. For preparation, solutions of 1.25% citric acid solution or 0.5% sodium carbonate solution, respectively, were added to the cocoons and heated for 30 minutes. Cocoons were immersed in an aqueous 8 M urea solution for 30 min., followed by 30 min. of refluxing at 85 °C for urea solution degumming. The authors came to the conclusion that urea solution and high temperature and pressure extraction of sericin produced better yields than the other techniques. Additionally, the sericin extracted using urea solution seemed to provide the most distinct protein bands on the SDS-PAGE. Sericin exhibits an endothermic breakdown at 220°C, which is greater than that of sericin produced using other techniques, indicating that the employment of chemicals during the extraction process affects the thermal stability of sericin. Other test results demonstrated that the sericin extraction procedure has the potential to alter the amino acid content and impact the chemical structure of proteins. The four techniques utilised in this investigation had four different effects on sericin conformation, although urea extraction had the most effect. However, in terms of tyrosinase inhibition, urea-extracted sericin had the strongest antityrosinase activity, whereas alkali-degumming had a little effect.

Other writers have also employed enzyme extraction. The discovery of cocoonase, a family of proteinases that may target the sericin binds, led to the use of enzyme to remove sericin from cocoons. The major kinds of enzymes employed for the degumming process were papain, trypsin, and bacterial enzymes . The peptide link between the carboxyl group of lysine or arginine and the amino groups of neighbouring amino acids is hydrolyzed by the proteolytic enzyme trypsin. Trypsin may readily hydrolyze sericin because of its comparatively high lysine and arginine content . Papain is a powerful cocoon degumming enzyme that acts with a broad selectivity towards polypeptides . Numerous fungal protease enzymes, including alkalase, a bacterial enzyme, have been standardized and shown to be commercially viable without posing any chemical risks . The many ways that sericin is extracted, as well as the origin and diversity of cocoons because of the various B strains. Mori offers several protein sizes, which are visible by changes in the content of amino acids and molecular weight , which may be reflected in biological qualities[11], [12].

CONCLUSION

The solubility in water is the only factor taken into account while removing sericin gum from crude silk . Usually, the protein comes from the B cocoon. yet, it may also be taken out of the silk gland. Soaps and detergents are often used in the conventional way of degumming silk , however this technique may cause sericin to undergo partial hydrolysis, losing some of its natural weight and functional qualities with the intention of removing and using the sericin

from B's cocoon. There are four ways to kill bacteria: urea, sodium carbonate solution, citric acid solution, and high temperature autoclaving, which may or may not be combined with high pressure . All of these techniques are adaptable in terms of temperature, duration, the chemical additive that was used, solution concentration, and other factors. Sericin makes up 25 to 30% of the weight of the cocoon and is a family of glycoproteins produced by alternate splicing of sericin genes . The expression of the genes is time-dependently controlled in accordance with the development of the larva, which increased some exon-exon homogeneity and produced a wide range of proteins .Sericin is produced by at least three genes: Ser1, Ser2, and Ser3. The first gene identified was Ser1, which is located at locus Src on chromosome 11 and has a single copy with about 23 kb and 9 exons. By using alternative splicing, Ser1 produces four large mRNAs that are respectively 10.5, 9.0, 4.0, and 2.8 kb in length. The Ser2 gene was found by Michaille et al. . It had 13 exons with sizes ranging from 28 to 2574 bp and encoded two mRNAs by alternative splicing. More complicated and varied than any other known gene generating silk proteins, the Ser2 gene was found to be. Because of the identical size of the first two exons that encode the signal peptides, its gene organisation mirrored that of the Ser1 gene Takasu et al. identified the final gene necessary for sericin production, the Ser3 gene, which is also found at chromosome 11, locus Src-2. This gene, which is about 3.5 bp in size and has three exons, encodes a straightforward transcript of 4.5 kb.

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CHAPTER 8
PROTEOMIC ANALYSIS OF LARVAL MID GUT FROM THE SILKWORM

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ABSTRACT:
The main organ for food digestion and nutrient absorption, as well as a barrier for external substances, is the midgut. For larval growth, development, and silk production, the fifth-instar larval stage of the silkworm is crucial. Genetic, Divergence, Implication of Diversity and Conservation of Silkworm

In all, 96 proteins were found in this research, 69 of which were discovered for the first time in the midgut. Additionally, we discovered that the silkworm larval midgut produced a 10 kDa heat shock protein and a precursor to the diapause hormone in response to hunger.The Bombyx mori silkworm has long served as a good model organism for research on the physiology of insects and the manufacture of silk. The larval midgut, which is essential to food digestion and absorption, is an important part of its biology. We performed a thorough proteome investigation of the larval midgut in order to get further understanding into the molecular pathways controlling these events. In this research, midgut tissues from silkworm larvae at different developmental stages were removed, and proteins were then isolated and examined using cutting-edge mass spectrometry methods. Numerous thousands of proteins were found at various developmental time periods as a result of our study, which indicated a dynamic and complicated proteome. These proteins included a broad variety of functional areas, such as those involved in the immune system, cytoskeletal proteins, transporters, and digestive enzymes. The varying needs of the larvae at several developmental stages were revealed by temporal profiling of the midgut proteome. Notably, we noticed that several proteases and lipases were upregulated throughout feeding phases, demonstrating their critical function in the breakdown of food proteins and lipids. Furthermore, the discovery of immune-related proteins pointed to a potential role for the midgut in host defences against infections. Additionally, the comparison of the midgut proteome across silkworm strains with various feeding habits and dietary needs revealed potential candidates for proteins that could aid in the silkworm's capacity to adapt to a variety of food sources.

KEYWORDS:
Instar, Larvae, Mid gut, Proteins, Silkworm,

INTRODUCTION

Bombyx mori, often known as the silkworm, is a tamed insect used to make silk. Due to its relatively large size and lab-reared status, the silkworm serves as a valuable model for structural and functional genomics as well as molecular genetics. The silkworm is a member of the lepidopteran class of nuisance insects and is also a phytophagous bug. The egg, larva, pupa, and moth are the four wonderfully distinct developmental stages of the silkworm, a holometabolous insect. The larva is the sole stage of the animal's life cycle during which it consumes food and excretes waste. The larvae have access to adequate nutrients during this stage, particularly the fifth instar, to support growth, development, and silk production. An epithelial cell monolayer made up of columnar, goblet, and stem cells forms the midgut of larvae[1], [2] .

The primary organs for food digestion and nutrient absorption are the columnar cells. Digestive enzymes typically regulate digestion and are reliant on their gut-specific localisation . Columnar cells have an apical membrane with a well-defined brush border . In the intestine, goblet cells help to regulate ions, and regenerative cells replace dying epithelial cells during metamorphosis .During the digestion of food, the midgut serves as another barrier to foreign particles. The midgut contains proteins with antiviral action against the Bombyx mori nuclear polyhedrosis virus , including lipase and SP-2 . Furthermore, the midgut has long been acknowledged as one of the key areas for controlling insects. Transgenic plants that generate B. thuringiensis crystal -endotoxins are one effective example. These poisons connect to their receptors and subsequently create an oligomeric prepore through which the insect dies as a result of the leakage of cell content . Using RNA interference via the midgut is an alternate strategy for more focused insect control . It may be used to silence gene expression.

Separately reporting the proteome study of the silkworm larval midgut, Yao et al. and Kajiwara et al. aimed to comprehend the molecular pathways for nutrition digestion and midgut-derived defence. The subsequent study concentrated on the midgut proteins from 5th-instar day-2 larvae whereas the earlier study only examined the 5th-instar day-3 silkworm larvae. It is well known that the development of the larval midgut in the fifth instar causes a significant shift in biochemical metabolism. We are investigating which proteins are involved in the development of the midgut in penultimate instar larvae. Additionally, when animals are starved, the animals will react violently by causing an emergent alteration in protein metabolism . Proteomic studies may show these as well. The midgut proteins of the 5th-instar larvae were examined in the current work using 2-DE in combination with MALDI-TOF-MS under both normal and starving circumstances. In the end, 96 proteins were found in this investigation, 69 of which were discovered for the first time. The outcomes also showed that in reaction to this unfavourable circumstance, the starving silkworm larva secreted a 10 kDa heat shock protein and a precursor to the diapause hormone[3], [4].

Preparation of Insects and Proteins

The Southwest University of China's Institute of Sericulture and System Biology donated the silkworm strain p50 that was utilised in this investigation. To reach the third day of the fifth instar, the larvae were raised on fresh mulberry leaves at 25°C. The larvae were then split into two groups at random. While group II's larvae were deprived until they reached the roaming stage , group I's larvae continued to be raised on fresh mulberry leaves. At 24-hour intervals, larvae were randomly selected from each group and dissected. The midguts were then cleaned with a 0.75% NaCl solution and kept at 20°C. Using a mortar and pestle and liquid nitrogen, larval midguts were pulverised to a powder. Using 1 mL of lysis buffer 3-1-propane sulphonate , and 4% dithiothreitol) at 4°C for 40 min, the proteins from silkworm midguts were extracted. The supernatants were collected for proteomics analysis after being centrifuged twice at 12,000 g for 15 min. According to the manufacturer's instructions, the Bradford kit was used to quantify the concentration of proteins[5], [6].

DISCUSSION

Using two-dimensional electrophoresis, the midgut proteins were separated in accordance with the manufacturer's instructions . In rehydration solution , proteins were briefly dissolved. An immobilised PH gradients strip was loaded with the protein solution. A total of 70000 Vhr worth of isoelectric focusing was carried out utilising the following voltages: 50 V for 12 hours, 100 V for 1 hours, 200 V for 1 hours, 500 V for 30 minutes, 1000 V for 30 minutes,

3000 V for 30 minutes, and 8000 V. The maximum current per strip was 50 mA. Following IEF, the strips were equilibrated for 15 minutes in equilibration buffer I then for a further 15 minutes in buffer II . A 12.5% SDS-polyacrylamide gel was loaded with the gel strip that had been equilibrated. In an Ettan DALTsix vertical electrophoresis system , electrophoresis was performed using 10 mA per gel for around 1 hour before switching to 40 mA per gel until the bromophenol blue reached the bottom of the gel. Silver staining was used to see protein.

Gel Image Analysis

The resulting 2D gels were scanned at an optical resolution of 300 dpi using GE Healthcare's Image Scanner III and LabScan 6.0 software. The ImageMaster 2D platinum 6.0 software that was provided by the manufacturer was used to analyse the scanned gels. The settings for spots detection were set at smooth: 2-5, minarea: 30-50, and saliency: 5-15.

MALDI-TOF-MS and In-Gel Digestion Analysis

Small portions of the protein spots that had been removed from the gels were broken up and placed into 0.5 mL Eppendorf tubes. In-gel digestion was performed in accordance with the methodology outlined in . In a nutshell, each tube received 100 L of the destaining solution 6], 50 mmol/L Na2S2O3) for a 10-minute destaining period. The gel bits were then rinsed three times for 15 minutes each with ddH2O. The gel bits were dried out using 100 l of ACN till they became opaque white. After centrifugation, the ACN was discarded. Over 40 minutes were spent drying the gel bits at room temperature. Each tube received 50 micrograms of trypsin before being incubated at 37°C for 16–20 hours. Twice with 30 L of 50% ACN/5% trifluoroacetic acid , the tryptic peptides were extracted. In a Centrivap Cold Trap , the supernatants were collected and dried. The peptides were redissolved in 3 L of 50% ACN/0.5% TFA prior to mass spectrometric measurement, and equivalent amounts of saturated -cyano-4-hydroxycinamic acid were added. On an Applied Biosystems Voyager DE PRO MALDI-TOF-MS , the peptides' mass spectra were captured. The database used in this analysis contained two types of protein sequences: 14623 sequences produced by the annotation of silkworm genomic sequences and 6642 sequences of silkworm proteins downloaded from NCBI with the keyword of Bombyx. According to a prior work , mass spectra were analysed and the protein identification procedure was completed[7], [8].

Real-Time PCR and RNA Extraction

Using the Trizol reagent , total RNA was isolated from the midgut tissues in two different groups. The concentrations of the RNA samples were determined using a spectrophotometer after they had been dissolved in RNase-free water. The first-strand cDNA was produced in accordance with the procedure using 3 g of total RNAs in a 25 L reverse transcription PCR machine using AMV Reverse Transcriptase for the diapause hormone precursor gene. In 20 L reactions using reverse transcription product, 2 PCR buffer, 50 ROX Reference Dye, 100 nM of each forward and reverse primer, and RNase free ddH2O, real-time PCR was carried out in triplicate for each gene of interest. The PCR reaction was carried out using the following programme on an Applied Biosystems ABI Prism 7000 Sequence Detection System: initial denaturation at 95°C for 10 sec and 40 cycles of 95°C for 5 sec and 60°C for 31 sec. The inner control was -actin3.

The fifth-instar day-4 to day-8 feeding larvae and the fifth-instar day-4 to day-13 nonfeeding larvae were used to extract the proteins from the silkworm midgut. According to Supplemental Data 1 and 2 in the Supplemental Material, a total of 15 extractions were divided using 2D technology. Following that, the protein spots were found using the

ImageMaster 2D platinum 6.0 programme. 439, 461, 498, 606, and 620 protein spots, respectively, were seen on gels V4 to V8. Protein spots were found on the gels V4N to V13N in the following numbers: 420, 455, 500, 610, 630, 635, 643, 640, 660, and 684. The number of protein spots steadily increased as the two groups of larvae grew. The protein spots on the V7 gel were identified by number and then examined using a Voyager DE PRO MALDI-TOF-MS, as illustrated in Figure 1.

Proteins having more than five peptide matches and peptide coverage more than 20% were allowed after being screened using GPMAW programme. Only the midgut of animals not receiving food had protein spots nos. 95 and 96. When compared to the data from the other research, our current analysis identified 69 proteins for the first time, making up 71% of all proteins. These 96 proteins were divided into 12 groups based on molecular function and GO annotation , including: Eight are used to store food, three are necessary for cell growth, sixteen are used for protein metabolism, three are used for carbohydrate metabolism, nine are used for lipid metabolism, eighteen are used for energy metabolism, three are related to the immune system, two are anti-oxidation proteins, eleven are related to muscles, five are used for hormone metabolism, sixteen are used for other purposes, and two are unannotated proteins. The discovered proteins have been submitted for GO annotation at http://silkworm.swu.edu.cn/cgi-bin/wego/index.pl. According to Figure 2, 96 proteins were divided into three categories: biological processes related to metabolism, cellular function, and biological control; molecular function encompassing binding, catalysis, transporter, electron carrier, and antioxidant; and cellular component[9], [10].

Modifications in Protein Pattern during the Growth of the Normal-Feeding Silkworm Larvae's Midguts

The proteins in the silkworm midgut varied from V4 to V8, as shown in Figure 1 and Supplemental Data 1. With the development of the larvae, the number of protein spots, particularly those with sizes between 30 and 90 kDa at higher pI regions, steadily increased., 64 midgut proteins, mostly those involved in storing food, protein/lipid/energy metabolism, and proteins associated to muscles, had varied abundances from V4 to V8. The thirteen proteins with differential expression differences more than twofold stood out the most. From V4 to V8, there were two locations that represented a juvenile hormone diol kinase and a fatty-acid-binding protein, with V6 showing the greatest expression level and 3). In V7, a protein that resembles an aberrant wing disc peaked). From Figures 3 to 3, one can see that ten proteins among which two were low-molecular-mass 30 kDa lipoproteins 19G1 and PBMHPC-19 precursor, three were ATP synthase subunits, and five were, respectively, protein disulfide-isomerase-like protein ERp57, enolase, arginine kinase, Enoyl-CoA hydratase precursor 1, and actin were expressed at relatively higher levels in V8. Table 1 also revealed that six proteins, including a 30 K lipoprotein precursor, an H+-transporting ATP synthase subunit, a cyclophilin, a glutathione S-transferase10, a NADPH oxidase, and an unidentified protein, were hardly discernible in feeding larvae prior to metamorphosis.

Proteins in the Gut in Response to Hunger

A portion of these fed silkworm larvae were segregated for starving after being fed on mulberry leaves for three days. The starving larvae managed to live for ten days. The majority of them perished from famine. At day 13 , some larvae were having difficulty pupating. Until day 13 , the midguts were meticulously removed from the starving larvae at 24-hour intervals. We next used 2D electrophoresis in conjunction with MALDI-TOF MS to further study these 10 protein samples, which ranged from V4N to V13N. Supplemental Data

2 revealed that, in comparison to V4 and V5, the protein profiles of V4N and V5N were relatively comparable, and the amount of protein spots on both gels reduced as starvation duration rose. However, compared to V5N, the spot count at V6N dramatically rose. The number of protein spots rose and ranged from 610 to 660 on the V6N to V12N gels. Given that V6N to V12N's protein distribution pattern approximated that of V5 to V8, it is possible that deprived larvae might maintain their midgut's structural integrity and functionality for a while. Also noteworthy is the fact that the number of spots rose at V13N, reaching 684. This likely occurred in conjunction with the remarkable transformation of starving larvae at that stage.

The proteins found in the midgut of starving larvae were compiled in Table 1. Table 1 demonstrates that more than 50% of the proteins discovered in non-feeding silkworm larvae followed a pattern of change comparable to that of feeding larvae. In contrast, 17 proteins take longer to manifest themselves than those in the feeding group. Additionally, before metamorphosis in feeding larvae, nine proteins that were still present in nonfeeding larvae were not identifiable; as an example, the expression pattern of actin-depolymerizing factor 1 Lepidopteran model organism and herbivorous insect is the farmed silkworm, Bombyx mori. The whole silkworm genome was sequenced by Chinese and Japanese researchers after tremendous effort , providing Bombyx researchers with the chance to detect peptides using the proteomic approach. For proteomic study, the midgut—the biggest digestive organ in the silkworm body—is crucial . The midgut of silkworms is yet another significant intra-extra interface, in addition to the cuticle. Pathogens, poisons, and pesticides may all enter via it. Therefore, understanding the protein components of the midgut of silkworms is crucial for creating novel lepidopteran pest management methods.

In this work, the midgut proteins of both the normal fifth-instar silkworm larvae and the starving larvae were analysed using two-dimensional electrophoresis in conjunction with MALDI-TOF-MS. From day 3 until the last day of the larva, the amounts of two categories of proteins steadily rose, which may indicate that an increasing number of midgut proteins are involved in the development and transformation of this tissue. 69 proteins among the 96 proteins found in this proteomics research were previously unrecognised , and the bulk of the midgut proteins found displayed developmental changes. For instance, JHDK is in charge of JH's deterioration. According to Li et al.'s research, JHDK mRNA was evenly distributed throughout the foregut and midgut of 4th- and 5th-instar larvae and expressed at a steady level throughout . Although JHDK protein in 5th-instar larvae reaches its peak at day 6, it is plausible that it may be the cause of the JH titer's decline at that point. Fatty acid transport is aided by the fatty acid-binding protein.

The gene for fatty-acid-binding protein was expressed in the 5th-instar silkworm larvae with a greater level during days 4-5, and subsequently reduced to a constant level, according to the microarray data. On our 2-DE profiles, the maximum abundance of this protein was seen at day 6, indicating that lipid metabolism was active at that time. At day 8, the amount of the aberrant wing-disc-like protein was considerably reduced. It participates in maintaining the integrity of the epithelium and contains nucleoside diphosphate kinase activity . Our finding suggested that hormones were in charge of controlling it. A transferrin was discovered as a result of our study. A protein called transferrin has several functions, including transporting iron, promoting cell development and differentiation, and protecting cells by preventing apoptosis .Different insects react to famine in different ways. Onthophagus taurus larvae react to food restriction by reducing the duration of the instar, becoming premature, pupating, and eclosing early to become tiny adults . In contrast to certain insects, such as Manduca sexta,

whose larvae would grow later when deprived . Bombyx mori is a member of the first group. The length of the larval stage would be extended if the 5th-instar silkworm larvae were deprived starting on day 3. It was discovered that Psacothea hilaris requires the threshold weight before transformation . This might be the cause of the majority of starving silkworm larvae failing to pupate.

According to our findings, nine proteins that were undetectable in feeding larvae before transformation were still present in silkworms who weren't eating.It's interesting to note that the deprived larvae generated more JHDK than the ones receiving regular nutrition. Lower JH titer with more JHDK. The final instar of the larva's metamorphosis is marked by a significant drop in JH. Although most of the starved larvae did not undergo metamorphosis, this finding suggests that the initiation of larval-to-pupal metamorphosis requires a low level of JH titer in combination with a high level of ecdysteroid titer in the nutrition stage. Due to digestive enzymes, the larval midgut is the primary location where digesting activity often takes place. In this investigation, we discovered that the digestive enzymes in the midgut of the larvae did not vanish under conditions of hunger. According to Santos et al these degraded enzymes, including glycosidase, amylase, and trehalase, were produced, secreted, and trapped in the glycocalyx. Since chemical pesticides have major negative effects on the environment, alternative pest control methods must be developed. There are now two main approaches for controlling pests using an insect's midgut . One is to lyse midgut epithelial using Cry toxins from B. thuringiensis as biocontrol agents. Utilising RNAi technology is another option. The mRNAs encoding vacuolar ATPase, ribosomal protein S4, actin, and -tubulin are among the chosen RNAi targets in the midgut . More midgut-derived pest control targets were offered by the current investigation[11], [12].

CONCLUSION

Actin-depolymerizing factor 1 is one of these proteins that regulates F-actin organization and cell and organ growth . Its role in the silkworm midgut will be discovered via more thorough investigation. Additionally, using proteomic analysis, we were able to pinpoint two proteins that were unique to the starving silkworm larvae's midgut. The other is a precursor to the diapause hormone, while the first is a little heat shock protein. Small heat shock proteins have been shown to function as molecular chaperones, refolding polypeptides caught in protein aggregates and destroying the mis-folded proteins . Small heat shock protein has been shown to have various roles, including the control of programmed cell death and the encouragement or suppression of apoptosis to preserve homeostasis . This tiny heat shock protein was not present in larvae that were fed normally, but it was present in those that were deprived, indicating that it may be necessary for the survival of starving larvae. Another protein found in the starved larvae is a diapause hormone precursor, which is connected to the manufacture of diapause hormone. Under severe circumstances, insects may enter a state of diapause. The expression of the gene that codes for the precursor to the diapause hormone suggests that when faced with famine, larvae might cease developing in order to survive

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CHAPTER 9
GENETIC, DIVERGENCE, IMPLICATION OF DIVERSITY AND CONSERVATION OF SILKWORM

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ABSTRACT:
Genetic variety is essential for the success of crop breeding in general because it tells us how much genetic difference there is and offers a foundation for particular breeding goals. One of the three types of biodiversity identified by the International Union for the Conservation of Nature as worthy of preservation. According to reports, humans tamed the silkworm, Bombyx mori, more than 5000 years ago to suit their needs. Greater genetic homogeneity in silkworms may increase their susceptibility to pests and illnesses, therefore genetic variety is a particular issue. Consequently, preserving genetic variation is a key element in long-term management methods for the genetic development of the silkworm, which is raised by millions of people worldwide for its luxurious silk. In this work, investigations on genetic variety in silkworms utilising various methodologies, as well as the current level of diversity and reasons causing diversity loss, are examined.

KEYWORDS:
Diversity, Genetic, Genotypes, Population, Silkworm.

INTRODUCTION

Because silkworms are raised on a large scale in rearing houses and their silk cocoons are used as fine fabric for garments, sericulture is a special branch of agriculture. Sericulture, like agriculture, needs a steady supply of productive silkworm breeds and host plant types to suit the constantly shifting demands of those working in the industry in addition to the consumer sector. Breeders must have access to an infinite variety of genetic resources in order to fulfil the constantly shifting demands from diverse industries. Because Bombyx mori is so economically significant, nations that produce silk, including China, Japan, India, Russia, Korea, Bulgaria, and Iran, have amassed a variety of silkworm breeds that are ideal for a variety of agroclimatic conditions. The germplasm of B is conserved with more than 4000 strains. Maintaining silkworm genetic resources, including univoltine, bivoltine, and polyvoltine strains, involves mori and 46 institutions. These many genotypes exhibit significant variations in both their qualitative and quantitative features, which ultimately regulate silk output. The silkworm genome is thought to be around 4.8′ 108 bp in size, or roughly one-sixth the size of the human genome. Currently, more than 450 morphological, physiological, and biochemical traits have been identified; of these, 300 have been found on 27 of the 28 chromosomal groups Along with a diverse range of regional races, B has a sizable population of mutants for many different types of characters [1], [2].

Silkworm Genetic Diversification

Research on genetic diversity is essential for crop breeding to be successful because it reveals the degree of genetic divergence and offers a foundation for particular breeding goals . The amount of genetic variation among individuals in a variety or population of a species is often thought of as genetic diversity . It is a result of the numerous genetic variations that exist between people and may take the form of variations in DNA sequence, biochemical traits

,physiological traits , or morphological characteristics . On the basis of various biometrical techniques, such as the phenotypic diversity index or coefficient of parentage using morphological, economic, and biochemical data, genetic diversity has traditionally been estimated [3], [4].

The variety of B's genes. Specifically, the strains with distinctive characteristics from Japan, China, Europe, and India were hybridised to create mori. In contrast to the tropical strains, which are resilient, tolerant of pathogen loads, and disease-resistant, temperate strains of silkworm generate more excellent, finer, stronger silk fibre overall. However, the tropical strains only generate little quantities of coarser, weaker silk . Divergence analysis approaches based on quantitative features have been presented to fit different purposes, aiding breeders in the process of identifying the parents that nick best. Multivariate statistical approaches have been used to assess the genetic diversity among the stocks since the majority of the desired characteristics in silkworms are of a quantitative nature. Among these, the study of Mahalanobis utilising Tocher's optimisation approach has a special position and is an effective way to determine the level of genetic diversity among genotypes, which quantifies the variation between various quantitative features. It has been discovered to be a very helpful technique for assessing genetic divergence in the silkworm and is employed by the majority of the workers . The genetic divergence investigation on silkworms that different researchers conducted is summarised in Table 1. By using this technique, the genotypes of silkworms were organised into several clusters, suggesting the occurrence of significant genotypic divergence. Despite the claimed existence of genotype divergence and the mixed tendency of clustering. In their examination of 49 silkworm breeds, Jolly et al. discovered that the breeds formed three separate clusters, demonstrating the existence of unique variation among the breeds. According to Subba Rao et al. and Govindan et al. , breeds descended from the same parents were grouped into several clusters, demonstrating the variance across breeds from the same source. However, breeds from the same source were found in the same cluster, demonstrating close kinship between advanced sister lines , whereas breeds from different genetic backgrounds were found in the same cluster, indicating consistency in the selection process . However, genotypes from temperate and tropical regions separated into distinct groups, demonstrating that the environment affects how characteristics are expressed . Although geographical variety is a potentially significant component, genetic divergence is not only determined by it . All of these studies sought to locate potential parents for breeding programmes and suggested crossing genotypes from various clusters to increase yield. Despite the fact that numerous silkworm characteristics have been the focus of divergence studies, only four characteristics—fecundity larval weight, single cocoon weight, cocoon shell weight, and filament length—contributed to the overall genetic divergence, which was roughly 97% .

The variety of silkworm

Most often, data that show variance in discrete allelic states or continuously distributed features are used to define genetic diversity, which gives rise to several genetic diversity metrics . Interspecific and intraspecific genetic diversity may both be measured among various accessions/individuals of the same species. relationship between families and genera . It is crucial to any breeding process, whether the goal is to take advantage of heterosis or produce useful recombinants. Any form of breeding plan places a premium on parent selection, therefore understanding the genetic diversity and relatedness of the germplasm is a must for crop development initiatives. Because a key tenet of natural selection holds that the quantity of genetic variety within a population is inversely correlated with its rate of

evolutionary change, genetic diversity is thus a crucial component of conservation biology . Because of a fall in fitness, populations are more at danger of extinction as genetic diversity decreases. Additionally, a variety of population, community, and ecological activities may be directly or indirectly impacted by genetic diversity. But in order for these effects to occur, genetic diversity must be linked to the degree of variation in phenotypic features .

The most common criteria used to distinguish between various genotypes of silkworms are the colour and structure of the cocoon, as well as larval, marking, and quantitative factors. Parents are then chosen depending on these qualities. However, the recent development of various molecular techniques has encouraged breeders to estimate genetic diversity based on information produced by various molecular markers. This has allowed for the quick analysis of germplasm and estimates of genetic diversity, which have been found to frequently support phenotypic data. These molecular markers may be roughly divided into molecular and biochemical markers[5], [6].

Biochemical indicators

More precise estimates of genetic diversity may be made using isoenzymes and other molecular markers than physical features. Variation at enzyme-coding loci are discovered by electrophoresis. Allozyme loci have the benefit of being codominant, making it easy to evaluate heterozygotes. It is well known that understanding an individual's genetic makeup in a population of races and allelic variants via isozyme research reflects the diverse catalytic capacity of allelic genes and their major contribution to the genotypes' adaptive strategy . Several publications have utilised isozymes to explore the variety of silkworm genotypes, including esterase, acid phosphatase, alkaline phosphatase, amylase, phosphoglucomutase, aspartate aminotransferase, malate dehydrogenase, glucose 6 phosphate dehydrogenase, and carbonic anhydrase . Due to its wide range of substrate selectivity and polymorphic expression, esterase was the most favoured isoenzyme among those that were examined . Four basic forms of esterase were discovered by Eguchi et al. ; around 70% of the examined Japanese, Chinese, and European races fall under the A type, 20% under the 0 type, and only the Chinese races exhibit the B type. Yoshitake et al.'s analysis of the esterase and acid phosphate polymorphism patterns in 300 different strains of silkworm revealed similarities between the distribution of these enzymes in the European and Japanese strains as well as between Chinese and European strains. On the esterase and acid phosphatase , there was more interstrain variability noted. Additionally, it has been shown that acid phosphatase is a useful marker for examining both intra- and interstrain diversity as well as strain differentiation . The findings of isozyme analysis performed on several silkworm genotypes by various authors revealed significant genetic variability among the genotypes, and they were mostly utilised to divide populations and strains in order to employ them in selection programmes [7], [8].

Chemical markers

Studies on molecular diversity evaluate all facets of genetic structure as well as complicated elements unique to each species . The identification of varietals and parentage helps in estimating genetic diversity and relatedness in germplasm, and the discovery and exploitation of naturally occurring DNA sequence polymorphisms has a broad range of potential uses in animal and plant development programmes . When employing biochemical markers like isozymes or morphological traits as markers, the outcomes may be significantly different from those obtained using various molecular markers. The farmed silkworm Bombyx mori has been successfully used to analyse the genetic diversity and phylogenetic relatedness using

the molecular markers RAPD, RFLP, ISSR, and SSR . Table 3 provides an overview of the diversity research done on silkworms using molecular markers. Two distinct groups—one consisting of diapausing genotypes and the other consisting of nondiapausing genotypes—were clearly distinguished by a RAPD-based dendrogram .

DISCUSSION

The "Chinese type" genotypes, which spin oval cocoons, were all clustered together among the diapausing genotypes, whereas the "Japanese type" genotypes, which spin peanut-shaped cocoons, were located in a different group. Additional genotypes that originate from the same region were clustered together in the same cluster . The nondiapausing silkworm genotypes that were created in India, China, and Bangladesh showed significant genetic variation, according to SSR and mtDNA markers study . Two separate groups, Khorasan native and Japanese commercial lines, were identified in the dendrogram created using RFLP markers. These two groups of strains and lines are clearly clustered, which indicates variations in their morphological, qualitative, and quantitative features as well as their geographic origins . Using the individual or nine concatenated intronic sequences, Kim et al. performed a phylogenetic analysis that revealed no clumping based on known strain characteristics such voltinism, moultinism, egg colour, blood colour, cocoon colour, or cocoon shape. Additionally, they reached a similar conclusion from the tree they created using the nine concatenated intronic sequences totaling 5,897 bp, including indels. However, according to Tunca et al. , the genotypes under study showed a somewhat low degree of variety. Jagadeesh Kumar recently revealed the low degree of genetic distance between the breeds based on gene frequency, which is supported by the boot strap values in the dendrogram that was created with the use of molecular markers. Overall, the phenotypic traits and molecular markers used in the diversity research had shown sufficient genetic variation between genotypes. However, these differences between the genotypes largely reflect voltinism and regional origin, revealing a small genetic basis.

Status of Silkworm Genetic Diversity

According to Zhang et al. , individual polymorphism in wild silkworm is substantially larger than that in domesticated silkworm, and genetic distances within Japanese strains are closer than those within Chinese strains. At the species level, Antheraea pernyi and Bombyx mori had significant levels of genetic variety, but Samia cynthia ricini displayed low levels of genetic diversity, according to Liu et al. . However, Antheraea pernyi showed the relative largest genetic diversity among the strains, and B. The least genetically diverse species was mori. Analysis of molecular variance revealed that strains in Antheraea pernyi and Samia cynthia ricini, respectively, had 60% and 72% of genetic variety, but strains in B only contained 16% of genetic variation. mori. Similar to this, the population size scaled mutation rate, which was much lower in domesticated strains of B than in wild strains of B , was used to evaluate genetic variation. mori. According to reports, domesticated strains exhibit heterozygosity at a rate that is twice as low as wild kinds . Quite low levels of DNA polymorphism were discovered recently by Yukuhiro et al. who examined PCR amplified gene segments for the enzymes carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase from 146 native strains of Bombyx mori. 42 samples of Japanese B. CAD haplotype study. Mandarin produced four distinct haplotypes. Between the two species, there was no shared haplotype, and at least five base substitutions were found. These findings imply little gene flow between the two species. Additionally, B has a very low degree of DNA polymorphism. the CAD gene itself or its closely associated areas

are potential candidates for silkworm domestication, according on comparisons between mori and its wild relatives. This information demonstrates the silkworm's limited genetic diversity[9], [10].

Causes of Genetic Diversity Loss

It is essential for a population to have genetic diversity if it is to be able to adapt to new environmental conditions. Genetically diverse populations need to develop morphological, physiological, or behavioural defences against the unfamiliar circumstances . In addition to producing populations that are more suited to their local habitats, this sorting procedure may, at least theoretically, result in a decrease in genetic variety. Effective population size, mutation, genetic drift, gene flow, inbreeding depression, outbreeding depression, and natural selection are factors that influence genetic diversity within populations. The domestication of silkworms, breeding practises, selection, genetic drift, and inbreeding may all have had a negative impact on the genetic diversity of the species. The main determinants of the quantity and distribution of genetic diversity in crop genomes relative to their wild progenitors in maize are selection and drift brought about by domestication.

Domestication

Humans have sampled, chosen, farmed, migrated through, and colonised new ecosystems throughout the last 12,000 years, which has resulted in a great deal of bottlenecks, drifts, and selection. By choosing desirable genotypes, plant breeders have sped up the process . With the earliest domestication of wild plants and animals, agricultural genetic diversity was altered and narrowed in the widest sense. After the Neolithic revolution, domestication took place over the course of the last 13,000 years, making it a relatively recent evolutionary event . In contrast to their wild relatives, domesticated organisms usually increase economically significant features and diversify physical qualities as a result of this process. An evolutionarily conserved morphogenetic gene may have undergone several changes and diversification as a result of silkworm domestication, a very recent evolutionary event . The genetic variety and homozygosity of the mulberry silkworm, which was domesticated more than 5000 years ago, are thought to have been drastically reduced as a consequence of domestication and selection. When comparing the full genome sequencing of 29 B, Xia et al. 11 Chinese B strains and mori strains. mandarina people by 1.50 billion quick readings and came to the conclusion that B. Mori and B were genetically distinct from one another. mandarina. At the same time, the authors calculated that a significant portion of B. Consequently, gene flow was restricted to B since mandarina individuals were employed for domestication . Numerous genes may have experienced damage during the domestication of silkworms. The degree of genetic variation in B has recently been found to be decreasing by Yu et al. and Guo et al. . in comparison to those in Chinese B, more genes or areas. the domestication-targeted gene in mandarin. Domesticated silkworms lost around 40.7% or 49.2% of the genetic diversity of wild silkworms .did a study using B. B and Mandarin. According to mori by Guo et al. , B is diverse. Compared to B, mori is substantially lower. mandarina. Additionally, all study techniques identified the artificial selection signature in the gene DefA, which may be subject to substantial artificial selection in B. Less variety was produced as a consequence of domestication . Nevertheless, when examining the genes for carotenoid binding protein in B. Large copy number changes and structural alterations linked to retrotransposons were found by mori in CBP compared to B. mori were not present in B. mandarina, and came to the conclusion that domestication may produce a large amount of variation in gene copy quantity and structure over a very short period of evolutionary time.

Breeding Also Reduces Variability

Breeding practises and life cycle characteristics control how genes are passed down across generations and have long been acknowledged as having an influence on genetic diversity and population genetic structure . Breeding has a significant impact on the loss of genetic variety ; introduction of contemporary variants is seen as a sign of genetic erosion . By definition, silkworm breeding is the process of gradually choosing better genotypes and/or phenotypes. A number of breeds have seen an acceleration in genetic improvement over the last several decades due to development and greater emphasis on more effective selection processes. As a consequence, native silkworm breeds have been supplanted globally by more productive ones. The deterioration of genetic resources is becoming a bigger problem as a result of this trend. Low-production breeds are important for preserving future breeding choices because they are likely to contribute to features of relevance now or in the future .

Natural selection causes the genotype's genetic foundation to become more restricted. Even if the breeder has added alleles from native races to his target genotype, he or she must now start the "weeding out" process of the undesired alleles. The genetic foundation of the line is once again becoming more restricted as unwanted alleles are eliminated. In reality, a breeder often takes the finest genotypes that are readily accessible and chooses better offspring. The population's genetic diversity tends to decline as a result of the continued use of the best genotypes as parents since this naturally reduces the gene pool to just those alleles that are accessible from the top parents . The replacement of natural species by alien high-yielding variants also poses a danger to the loss of genetic diversity. Usually, the size of the population is a significant factor in genetic diversity loss.

Selection's Impact on Diversity

Any population's patterns of variety are likely to be impacted by selection. Varieties may be maintained in populations through balancing selection brought on by overdominance or by frequency dependent selection, and environmental variations may select for various genotypes in various populations . However, purifying selection eliminates harmful variations that result from mutation; these variants are anticipated to occur at lower frequency than anticipated for the neutral equilibrium. When favorable mutations quickly reach high frequencies, whether they spread across a species to fixation or merely within a population undergoing adaptation to its local environment, it results in another kind of directional selection . In the breeding projects for B, artificial selection has been used extensively. mori, a significant insect for commerce. Nevertheless, with systematic broad selection for a small number of desired features, the genetic diversity of silkworms is significantly decreased. The selection of better individuals often leads to genetic gain but also a loss in genetic diversity and is highly influenced by the technique and degree of selection. Two significant effects of selection will occur: a change in the genetic average value, which is traditionally evaluated as a gain; and a change in diversity, which will be measured by the relative effective number of families. It is well knowledge that directional selection has an impact on variety. This research suggests that selection and inbreeding might lead to lines that are separated from the original parental populations with differing genetic characteristics. Continual selection and inbreeding, in which shorter larval length is the dominant and fitness characteristic, may have caused a homozygous condition of the recessive gene for prolonged larval duration, according to Strunnikov . Despite introducing variety, its prospects of survival are compromised since it lost its dominating or fit personalities. Furthermore, according to Seidavi , selection based on productivity traits resulted in poor survival due to the genetic

performance of the selected population of silkworms for the cocoon weight trait after the fourth generation, indicating the impact of selection on diversity.

Biological Drift

Genetic drift, which happens when gametes are randomly chosen from one generation to the next in a limited population, is a chance shift in allele frequency. On each locus in the genome, it has the predicted impact . Genetic drift often only results in a minor chance shift in the allele frequency in a large population. On the other hand, if the population size is small, the allele frequency might change significantly over time in a manner that seems random, leading to the chance fixation or loss of an allele. Buri offered a well-known example of how a limited population size impacts allele frequency. In randomly chosen populations of size 16, he examined the frequency of two alleles at the brown locus, which determines the colour of the eyes in Drosophila melanogaster. However, at various generations, 107 or so groups contained 0 to 32 bw75 genes. After generation 4, the total number of populations fixed for one of the two alleles grew almost linearly, and by generation 19, the number of populations fixed for the two alleles was almost equal, with 30 populations fixed for bw and 28 fixed for bw75. In silkworm germplasm maintenance centres, only 40–60 cocoons from each strain or breed are chosen at each cycle for the next generation; from these, only 20 layings are prepared, and only 5–6 layings are subsequently brushed. Due to the tiny population size, Buri theorised that allele frequency may vary[11], [12].

CONCLUSION

Eventually, directional or disruptive selection will fix one allele, eliminating genetic variety. Because canalisation may be prevented by directional selection and may favour mechanisms that increase phenotypic variation , it has been hypothesised that directional selection lowers the level of developmental precision or stability . Systematic parental selection also reduces genetic variation among offspring. The decrease will stabilise after 4-5 generations with the same level of selection. According to Pradeep et al. , the silkworm is a textbook illustration of how inbreeding and directional selection may alter diversity. Based on the shortest larval duration and the longest larval duration , they divided the larval populations of the Nistari strain and maintained them for four additional generations. Polymorphic profiles were produced in LLD and SLD lines using RAPD and ISSR primers. From the third generation on, distinct markers that were unique to LLD individuals were seen, indicating that selection had caused allelic variations to differentiate for a longer larval period

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CHAPTER 10
EXPRESSION AND FUNCTIONAL ANALYSIS OF STORAGE PROTEIN IN THE SILKWORM

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ABSTRACT:
Storage protein 2 inhibits cell apoptosis in addition to serving as a vital source of energy for silkworm growth and development. A study of SP2's antiapoptotic effect on endothelial cells may help with the treatment of atherosclerosis and other cardiovascular illnesses since EC apoptosis is a significant role in the development of atherosclerosis. In this investigation, the sp2 gene was cloned, the Escherichia coli product of which was a 6xHis-tagged fusion protein from which a polyclonal antibody was produced. The SP2 levels of fifth-instar larvae were greater in the pupal stage and hemolymph, but were lower in the egg and adult stages, according to the findings of a western blot. The findings of subcellular localization indicated that SP2 is mostly found on the cell membrane. In addition, a recombinant baculovirus for SP2 expression was created using a Bac-to-Bac method. After that, staurosporine-exposed human umbilical vein ECs in a culture medium were given the pure SP2. SP2 might considerably improve HUVEC vitality, according to a cell viability study. Additionally, the findings of ELISA and flow cytometry demonstrated that SP2 inhibits the apoptosis that staurosporine-induced HUVEC undergoes.

KEYWORDS:
Analysis, Apoptosis, Cells, Proteins, Silkworm

INTRODUCTION

The blood artery walls' vascular smooth muscle and the flowing blood are connected at the cellular level via the vascular endothelium. Additionally, it plays a crucial part in maintaining the equilibrium of the endovascular environment. Reactive oxygen species are produced by NADPH oxidase in endothelium and vascular smooth muscle as a result of a variety of factors, including peroxide, oxy-low density lipoprotein , angiotensin I, and tumour necrosis factor . This oxidative stress causes endothelial cells to undergo cell apoptosis. Atherosclerosis and other cardiovascular disorders may be brought on by EC dysfunction. Numerous investigations have shown that one of the many atherogenic causes is EC apoptosis .Additional research has shown that silkworm hemolymph may prevent the death of insect and human cells that is brought on by viruses and a number of chemical inducers, including staurosporine, camptothecin, and actinomycin D . The 30 K protein is one such antiapoptotic element found in silkworm hemolymph, and it has received much research[1], [2] .

.Animals, cell lines, strains, and reagents

LB medium was used to culture the Escherichia coli strains TG1 and BL21 at 37°C. In our lab, the pFastBac HTB vector and pET-28a expression vector were both constant. The 10% foetal bovine serum -supplemented TC-100 medium was used to sustain the silkworm-derived cell line BmN at 27°C. In BmN cells, the B. mori nuclear polyhedrosis virus was multiplied. HUVECs were acquired from the American Type Culture Collection in Manassas, Virginia, and grown in Dulbecco's modified Eagle media with 10% FBS in a humid incubator

at 37°C in a 5% CO2 environment. Fresh mulberry leaves were used to raise fifth-instar silkworm larvae at a temperature of 25 C. We bought male New Zealand rabbits from the Hangzhou Normal University's Animal Research Centre. Promega sold a DNA gel purification kit and goat anti-rabbit IgG that was labelled with Cy3. IgG goat anti-rabbit that has been HRP-labeled was bought from Dingguo Biotechnology in Beijing, China. Invitrogen provided the Cellfectin II Reagent and the Ni-NTA Purification System; Roche Diagnostics provided the Cell Death Detection ELISA kit; Invitrogen provided the Annexin V-FITC/PI apoptosis detection kit; and the Beyotime Company provided the staurosporine. Other reagents were analytical-grade goods that were acquired locally[3], [4].

Bioinformatics analyses

GenBank's BLASTn and BLASTp algorithms were used to compare the nucleotide and protein sequences. On the ExPASy website , the hydrophobicity analysis and the isoelectric point prediction were carried out. The SWISS-MODEL programme was used to simulate the protein's tertiary structure.

Making polyclonal antibodies

The sp2 open reading frame was amplified by PCR utilising a template made from the silkworm pupa cDNA library developed by our group. The forward and reverse primers included BamHI and XhoI restriction sites, respectively, and were as follows: The expression vector pET-28a was subcloned with the sp2 gene in it. The recombinant plasmid was used to convert Escherichia coli BL21 , which was then grown in LB medium with 50 g/mL kanamycin at 37°C until the OD600 value reached around 0.5. 1 mmol/L IPTG was used to promote the expression of recombinant protein for 4 hours. The bacteria's His-tagged fusion protein was removed, and it was then cleaned using Ni2+-affinity chromatography. After that, dialysis was used to concentrate and desalt the purified protein. In order to create the polyclonal antibodies, the protein content was assessed using the Bradford technique before being utilised to immunise the male New Zealand rabbits. The titer of the polyclonal antibody was measured using an ELISA, and the specificity of the polyclonal antibody was discovered using a Western blot analysis[5], [6].

DISCUSSION

The distribution of SP2 in various tissues of the fifth-instar larvae and later silkworm developmental stages was assessed using a Western blot analysis. The head, epidermis, hemolymph, fat body, silk gland, trachea, Malpighian Tubule, and midgut tissues of fifth-instar larvae were separated and ground into powder in liquid nitrogen. The tissues were then pulverised and the mixture was reconstituted in a lysis buffer for 30 minutes. At 4°C, the homogenates were centrifuged for 15 minutes at 12,000 g. By measuring the protein content , the total protein-containing supernatants were equalised. The samples were examined by electrophoretically transferring them onto polyvinylidene difluoride membranes and putting them through a 12% SDS-PAGE. After blocking the membranes overnight at 4°C with 3% skim milk in phosphate-buffered saline , rabbit anti-SP2 antibody was added, and the mixture was allowed to sit at room temperature for 2 hours. The bound antibodies were identified by anti-rabbit IgG followed by a DAB detection method after washing with PBST [7], [8].

SP2 Localization in Subcellular Environment of BmN Cells

On a glass coverslip that could be used particularly for confocal imaging, BmN cells were grown for the night. The cells were then rinsed three times in PBS for 5 minutes each, fixed

in 4% polyformaldehyde in PBS at room temperature for 15 minutes, and then permeabilized in 0.2% Triton X-100/PBS for 10 minutes. The anti-SP2 polyclonal antibody was diluted 1: 1000 in blocking buffer and incubated with the fixed cells at room temperature for 2 hours. The cells were also incubated with control serum, which was obtained from rabbits before they were immunised with the antigen. Cells were treated with Cy3-labeled goat anti-rabbit IgG for 2 hours at 37°C before being washed twice in PBST. After that, the cells were kept at 37°C for 30 min while being treated with 4-6-diamidino-2-phenylindole . The cells were imaged using a Nikon Eclipse TE 2000-E Confocal Microscope after being rinsed with PBST once. EZ-C1 software from Nikon was used to analyse the images.

Expression of SP2 in a Bacto-Bac System

Between BamH I and Xho I sites, the sp2 gene was inserted into the MCS of the transfer plasmid pFastBac-HTB before being converted into DH10Bac cells. The recombinant baculovirus bacmid-sp2 was created by homologous recombination after the sp2 gene was inserted into a wild type bacmid DNA. The positive colonies were chosen after white-blue plaque selection, and they were then analysed by PCR using M13 universal primers and sp2 forward and reverse primers. For amplification, the recombinant bacmid was transfected into BmN cells. Further BmN cells were infected with the third-generation virus for protein expression. The cells were grown for 48 hours before being collected using washes in phosphate buffer solution , pulsed ultrasonography, and centrifugation at 12,000 rpm for 10 minutes. Ni2+-affinity chromatography was performed on the supernatant as directed by the product's manufacturer . The pure fusion protein was found using SDS-PAGE and Western blotting. BmN cells that were infected with wtbacmid were utilised as a control. The SP2 protein was concentrated after affinity chromatography purification, and imidazole was eliminated using dialysis against 25 mM HEPES at pH 7.5 and 100 mM NaCl. The Bradford technique was used to determine the protein content.

The induction of apoptosis in HUVEC

After being separated using 0.25% trypsin, HUVECs were grown in Dulbecco's modified Eagle medium , which was supplemented with 10% FBS. The cells were kept at 37°C in a humidified environment with 5% CO2 until they achieved 70-80% confluence. An initial concentration of 0.5, 1, and 2 g/mL of culture media containing pure SP2 protein was applied to cells for a 24-hour pretreatment period. Negative and typical controls were left unaffected. The normal group was given 2 mL of Hank's balanced salt solution after being washed twice with it. The experimental and negative groups were then both exposed to 2 mL of STS for 2 hours to cause apoptosis. After two rounds of washing with Hank's balanced salt solution, the experimental group was cultivated in culture media with pure SP2 protein present . After 12 hours, HUVEC viability and apoptosis were assessed.

Cell Viability Analysis MTT Assay use

Using the MTT test, cell viability was calculated. One 103 cells per well were planted onto 96-well plates, which were then left to incubate overnight. 10 L of MTT was added to each well after the treatments as described above and incubated for 4 hours. To determine the relative cell viability ratio, the insoluble formazan crystals were dissolved in 200 L/well dimethylsulfoxide, and absorbance was measured at 490 nm. At least three times each experimental condition was repeated.

Finding DNA Fragmentation

According to the manufacturer's recommendations, DNA fragmentation was assessed by histone-associated DNA fragments using a photometric enzyme immunoassay . At least three times each experimental condition was repeated.

Analysis via Flow Cytometry

Harvested cells were washed twice with ice-cold PBS after being plated at a density of 1 106 cells/mL. According to the instructions provided by the manufacturer , staining with Annexin V-FITC and PI labelling was carried out before flow cytometric analysis. Using a FACSCalibur , FACS analysis was carried out.

Statistical Analysis

The Student's -test and the Newman-Keuls test were used to analyse the data, which were reported as mean SEM. Statistics-wise, differences having a value of were deemed significant.

Silkworm SP2 Polyclonal Antibody Production

The prokaryotic expression vector pET-28a was subcloned with the sp2 ORF. In E. coli BL21 , the His-tagged fusion protein was produced, and it was then isolated using Ni2+-affinity chromatography). SDS-PAGE effectively identified the isolated His-SP2 fusion protein). The estimated result is consistent with the expected molecular weight of the fusion protein, which was 87 kD and included a 3.56-kD His-tag. Using the Bradford test, it was found that the purified SP2 protein concentration was 1.5 mg/mL. By immunising a male New Zealand rabbit with the affinity-purified proteins, anti-SP2 polyclonal antibodies were produced. According to ELISA, the polyclonal antibody's titer was 1:6400. According to a Western blot examination, the antibody only interacted with purified His-SP2 fusion protein.

Expression Analysis of Silkworm SP2

Western blot analysis was carried out on protein extracts to ascertain the distribution of SP2 in various tissues of the fifth-instar larva and further silkworm development stages. According to the findings, SP2 levels were remarkably high in pupal and larval stages but remarkably low in egg and adult stages). The hemolymph and fat body of the fifth instar larvae had the greatest SP2 levels, whereas the trachea and midgut had the lowest levels.

SP2's Subcellular Localization

The Nikon ECLIPSE TE2000-E Confocal Microscope was used to study the treated cells, and EZ-C1 software was used to analyse the pictures. When triggered with 353 nm light, DAPI-stained nuclei flash red, and when stimulated with 550 nm light, goat anti-rabbit IgG is labelled with Cy3. According to our findings, SP2 is mostly found in the cell membrane and just little in the cytoplasm[9], [10].

SP2 Is Expressed and Purified in a Bac-to-Bac System

Recombinant bacmid-sp2 was utilised to infect BmN cells in order to produce the His-tagged protein while wtbacmid was employed as a control in order to evaluate the function of SP2 protein). The findings of the Western blot did not reveal the particular band in the BmN cells lysates infected by wtbacmid due to the low expression level of natural SP2 protein in BmN cells). The BmN cell lysates were chromatographically separated using Ni2+-affinity. Using

the Bradford test, it was found that the purified SP2 protein concentration was 0.108 mg/mL. The isolated fusion protein SP2 had a molecular mass of around 87 kD, according to the findings of SDS-PAGE and Western blot analysis and 5).

HUVEC Apoptosis Induced by STS and Apoptosis Assay

Internal DNA fragmentation, a sign of apoptosis, and morphological alterations are both triggered by STS in the cell . To investigate the impact on HUVEC apoptosis, STS was administered at escalating concentrations. Using ELISA, apoptotic cells with DNA fragmentation were examined 24 hours after STS treatment. Apoptosis was unaffected by STS at low concentrations , but it caused necrosis at high concentrations , and DNA fragmentation at 1 M was effectively activated in comparison to the kit's positive control, camptothecin. On the basis of these findings, 1 M STS was used in the tests that followed. Purified recombinant SP2 protein was added, and this improved cell vitality in a dose-dependent manner , suggesting that SP2 may increase apoptotic HUVEC viability. An ELISA was utilised to measure DNA fragmentation and study the inhibitory impact of SP2 protein on STS-induced HUVEC apoptosis. As shown in Figure 7, recombinant SP2 protein dramatically and dose-dependently reduced DNA fragmentation, a marker of HUVEC apoptosis. Using a flow cytometry analysis and an Annexin V-FITC/PI apoptosis detection kit, the number of apoptotic HUVECs was determined. Fluorescein conjugated to human anticoagulant Annexin V, which was able to attach to phosphatidyl serine in the outer leaflet of the membrane of apoptotic cells, was used to identify these cells. Propidium iodide was added before to examination in order to identify cells that had lost membrane integrity. Figure 8 demonstrates that whereas the proportion of apoptotic HUVECs that had received 1 M STS treatment was 52.9%, the apoptosis rate of untreated HUVECs was 1.9%. The apoptotic rate of HUVECs treated with STS + SP2 dropped to 42.1% when compared to the negative control. These findings suggest that SP2 has anti-apoptotic effects on STS-induced HUVEC death.

The endothelium, which serves as the primary regulator of vascular homeostasis, has a number of vasoprotective effects, including vasodilation, reduction of smooth muscle cell development, and suppression of inflammatory reactions. In the pathological condition of atherosclerosis, EC apoptosis plays a significant role. Superoxide and other ROS have been implicated in endothelial dysfunction, according to a significant body of data. Antioxidant vitamins C and E may help endothelial function return, which will slow the development of atherosclerosis . Other elements may also prevent EC apoptosis, including vascular endothelial growth factor , low serum nitric oxide levels, calcium antagonists, prostacyclin, and oestrogen . Numerous clinical studies have been conducted to examine the effectiveness of anti-apoptotic genes and proteins in the treatment of disorders linked to apoptosis. Apoptosis-related disorders may benefit from the use of silkworm hemolymph and its 30 K protein, which have been shown to have anti-apoptotic effect in a variety of human and insect cell systems. Another possible anti-apoptotic protein in silkworm hemolymph was investigated in the present work.

The SP2 protein, a member of the hemocyanin superfamily, performs a variety of biological tasks . Many species of arthropods and mollusks have hemocyanins, which are big copper-containing proteins that carry oxygen in the hemolymph . The majority of hexamerins are thought to be store proteins that act as carriers for other proteins or as sources of energy. They may also play a role in the humoral immune response. As a result, they are regarded as a crucial immunological molecule in arthropods. Given the significance of hexamerins in the

development of arthropods, we hypothesised that silkworm SP2 protein may likewise play a significant role in cell death. The SP2 protein was produced and purified in both a prokaryotic expression system and a silkworm baculovirus system in order to investigate its activities. The distribution and location of SP2 in silkworms were studied using monoclonal antibodies made from the pure SP2 prokaryotic expression product, whilst the antiapoptotic effect of SP2 was investigated using the purified expression product.

Our investigation of the levels of SP2 in various tissues from the fifth-instar larva and later silkworm developmental stages revealed that the hemolymph and fat body had the greatest amounts of SP2, whilst the egg and adult stages had the lowest levels. These findings imply that hormones related to puberty and moulting have an impact on the sp2 gene's activity . According to our investigation of SP2's subcellular localization, SP2 was present in the cytoplasm and cell membrane of the BmN cell line, although it was mostly located there. Our findings imply that SP2, a secreted protein with a signal peptide predicted by bioinformation , is transported across the cell membrane under the guidance of the signal peptide. We created an STS-induced HUVEC apoptosis model to investigate SP2's anti-apoptotic action on vascular EC death. ROS production, which causes oxidative stress on the cells, was induced by the STS treatment. In cultured HUVEC, STS at a concentration of 1 M significantly increased apoptotic effects. The pure recombinant SP2 protein was able to dramatically increase HUVEC vitality and decrease HUVEC apoptosis brought on by STS, according to an MTT test, DNA fragmentation detection, and flow cytometric analysis. These discoveries provide fresh perspectives on how to avoid endothelial dysfunction and may eventually lead to atherosclerosis treatments[11], [12].

CONCLUSION

Storage protein 2 , a different protein found in silkworm hemolymph, has also been shown to be able to prevent staurosporine-induced ROS production and HeLa cell death . The fat body of feeding larvae produces SP2 and releases it into the hemolymph under the influence of juvenile hormone and ecdysone . It is preferentially reabsorbed by the fat body cells at the conclusion of the feeding phase . The amino acids necessary for the growth of adult tissues are thought to be stored in SP2 .In the present work, the silkworm SP2 gene was cloned and expressed in both a prokaryotic expression system and a silkworm baculovirus system. To produce polyclonal antibodies, Escherichia coli produced protein was employed. Western blot was used to find SP2's distribution across several tissues and developmental stages. In addition, the anti-apoptotic effects of SP2 on human umbilical vein ECs triggered by peroxidation were investigated using the expressed protein in the silkworm baculovirus system. This research will set the groundwork for the production and use of protein medicines derived from commercially significant insects for the treatment of vascular disorders

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CHAPTER 11
MICROARRAY ANALYSIS OF THE JUVENILE HORMONE RESPONSE IN LARVAL INTEGUMENT OF THE SILKWORM

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ABSTRACT:
20-hydroxyecdysone and juvenile hormone work together to control insect larval development and moulting. About how this cooperative control is implemented throughout the larval stages, nevertheless, nothing is known. Here, we employed the JH analogue metho-prene to induce silkworm superlarvae using a microarray technique to examine the mRNA expression changes in response to JHA in the silkworm integument. In the integument, we discovered that JHA treatment considerably elevated the expression levels of the majority of genes involved in fundamental metabolic and protein-processing functions while lowering the expression of genes related to oxidative phosphorylation. After JHA administration, many important genes involved in the insulin/insulin-like growth factor signaling and 20E signaling pathways were also elevated. Together, our findings imply that JH may affect 20E signaling and multiple metabolic pathways to mediate the nutrient-dependent IIS pathway.

KEYWORDS:
Expression, Genes,Larval, Microarray, Silkworm.

INTRODUCTION

A sesquiterpenoid hormone called juvenile hormone works in conjunction with 20-hydroxyecdysone to control a variety of physiological processes in insects, including growth, development, and reproduction . JH is often produced by the corpora allata in insects and aids in maintaining larval development.Ecdysone, which is generated in the prothoracic glands, is converted into 20E, which causes metamorphosis and larval moulting during the larval-pupal transition. While lower levels or a lack of JH at the end of the larval or pupal stages permits 20E to induce metamorphosis, higher levels of JH during larval stages hinder metamorphosis. In mature insects, JH controls the development of the female reproductive system [1], [2].

A intriguing topic is what mediates the JH response. The transcription of several genes may be induced by JH in vivo or in vitro, according to some recent research, and unique JH-response elements in certain JH-regulated genes have been discovered . The genome-wide response to JH and the interactions between JH and 20E in insect larvae are both poorly understood. It's significant to note that a transcription factor called Krüppel homolog 1 is transcriptionallycontrolled by JH and functions as a repressor of insect metamorphosis by transducing the JH signal. It has been established where Kr-h1's binding motif is located in the target's promoter. Kr-h1 is thus recognised as a very accurate marker of insect JH sensitivity.

In general, larval phases are when insects eat and develop. JH has a role in keeping insects at the larval development stage, which is consistent with its role in regulating metabolic balance in insects. For instance, preliminary research on the silkworm suggested that the administration of JH analogue reduces ATP production . According to recent studies, JH controls trehalose homeostasis and lipolysis in insects . It's interesting to note that the nutrition signalling route during insect development is likewise mediated by the

evolutionarily conserved insulin/insulin-like growth factor signalling pathway . In the Aedes aegypti, a mosquito that transmits yellow fever, the IIS pathway takes role in the dietary control of JH production . In addition, JH regulates the production of insulin-like peptide 2 in the red flour beetle to alter trehalose homeostasis and starvation resistance . We know very little about the JH-regulated metabolic pathways and how they relate to the IIS pathway.In the early stages of each larval instar, the JH titer is high, but the 20E titer is raised in the later stages. This variation points to a crucial interaction between JH and 20E. Several molecules, including Met, EcR, Broad complex, and Kr-h1, have been shown to coordinate the crosstalk between JH and 20E throughout insect growth and development, to the best of our knowledge . Interesting research has shown that the IIS pathway controls ecdysteroidogenesis . These results suggest that JH may influence 20E levels through regulating the IIS pathway's activity, which is nutrient-dependent[3], [4].

The clear distinction between eating and moulting throughout the larval stages makes the silkworm a great model for researching crosstalk between JH and 20E. Several papers highlight the activities of JH in silkworms. JH, for instance, has been shown to be a strong activator of PTTH secretion, which is a peptide that encourages ecdysteroidogenesis . Precocious metamorphosis and a reduction in larval moulting times are caused by overexpression of the juvenile hormone esterase gene, which is involved in JH metabolism, mutation of the Cyp15C1 gene, which is involved in JH biosynthesis, or direct downregulation of JH biosynthesis in the silkworm . The emergence of superlarvae with an extra larval instar is flawlessly induced by the administration of JHA methoprene at the start of the fourth larval instar . JH is also implicated in the immunological response and glycolysis . The response of the whole silkworm genome to JH and the genetic underpinnings of the interactions between JH and other signalling pathways, such as the 20E signalling and IIS pathways, are yet unknown.

We looked at the changes in gene expression throughout the genome after JHA administration to understand the genomewide response to JH in vivo and to discover fresh hints to the impact of JH on the signalling pathways relevant to larval growth and development. We used a silkworm microarray and a model of silkworm superlarvae produced by JHA application to evaluate gene expression in the integument of the silkworm. Our findings demonstrate that JHA treatment raises the expression levels of genes related to the IIS system and numerous metabolic activities in the silkworm[5], [6].

Strain of Silkworms

In our studies, the tetramolter silkworm strain Dazao , which undergoes four moults while in the larval stage, was employed. The larvae were raised at 25°C with a 12-hour light/12-hour dark photoperiod on fresh mulberry leaves.

Superlarval JHA Induction of Silkworm

One hour after the third larval moult was finished, we removed individual larvae and raised them together for hormone therapy studies. The JHA methoprene was dissolved in acetone . The freshly moulted fourth instar larvae received dosages of 10–30 g per larva along the dorsal midline of the larval thorax, according to other reports . As a control, silkworm larvae that received the same dosage of pure acetone were employed[7], [8].

Analysis Using Microarray

Insect larval integument, which includes muscle, is regarded as a peripheral tissue that JH targets . We separated the integument from 20 larval individuals at various time periods following JHA treatment, including 12 hours after treatment with JHA or acetone , 24 Hat, 36 Hat, and 48 Hat. This allowed us to examine the genome-wide reactions to JHA application in the silkworm integument. At each time point, samples of the skin were taken, snap-frozen in liquid nitrogen, and kept at 80 °C. As biological duplicates, three distinct samples were isolated for each time point.

Using the silkworm genomewide microarray, which has been extensively utilised to profile gene expression in various research , we further examined the changes in gene expression after JHA treatment. Following the procedures outlined in other studies , total RNA extraction, microarray hybridization, raw data normalisation, and microarray data analysis were carried out. In a nutshell, the produced sample was treated with TRIzol reagent to extract the total RNA. CapitalBio Corp. carried out the normalisation of raw data and microarray hybridization. We combined equal quantities of total RNA from three biological replicates at each time point to form one sample for the hybridization experiment due to the high cost and great repeatability of microarray hybridization . Each mixed RNA template was used to create cDNA for the microarray hybridization, which was then applied to the silkworm genome oligonucleotide microarray and labelled with fluorescent dyes . Each hybridised array's picture was scanned to collect the raw signal intensity data for gene expression, which was then normalised to the known housekeeping genes [9], [10].

When analysing the microarray data, we came to the conclusion that a gene was expressed if its expression signal strength was more than 400 units in any sample at any time point. By comparing it to the control, the ratio of a gene's expression change at each time point following JHA administration was assessed. The ratio of a gene's expression change at each time point was then utilised to profile the gene's expression change across time. A gene was deemed to have undergone up- or down-regulation after JHA treatment if its expression level changed by at least 2.0-fold in comparison to the control. These genes with differential expression were all classified as JHA-modulated genes. The Cluster 3.0 programme was used to achieve hierarchical clustering of gene expression . We used a coexpression analysis using the GeneCluster 2.0 programme to examine the parallel changes in gene expression of the JHA-modulated genes throughout four chosen time periods . The GEO database has received all of the microarray data used in this work and has assigned accession number GSE53374.

The KEGG Pathway Analysis and GO Annotation

We functionally inferred the gene ontology terms of the JHA-modulated genes using the online WEGO programme . Additionally, we conducted a BLAST search against the silkworm gene collection using the probe sequences of all up- and downregulated genes to identify the genes that matched the probes. The KAAS programme allowed these probe-matched genes to be mapped to several KEGG pathways utilising the online KEGG pathway database and sequence similarity .

Experiment with Quantitative Real-Time RT-PCR

We investigated the alterations in the mRNA expression of certain genes in the integument at 12, 24, 36, and 48 hours following JHA treatment using quantitative real-time RT-PCR assays. After applying JHA, we examined the expression of the same gene collection in the fat body, another JH-targeted tissue, for a second comparison. We only dissected the fat body

at 36 and 48 hours after JHA treatment since it is challenging to do so at the early stages of the fourth larval instar. There were three biological duplicates. Following previously mentioned protocols, sample collection, RNA extraction, and cDNA synthesis were carried out .The SYBR Premix Ex Taq system was used for real-time RT-PCR. Each PCR experiment used a final volume of 20 L, 70 ng of cDNA in 2 L, 10 L of SYBR Premix Ex Taq, and 0.4 M primers. The following PCR amplification settings were chosen: 40 cycles of 95°C for 30 s and 60°C for 30 s each. The translation initiation factor 4A gene was used as a reference point to normalise the mRNA expression levels of the chosen genes. Table S1 contains a list of all the primers used in this investigation. Further calculations were made to determine the Pearson correlation coefficient between the microarray data and real-time RT-PCR findings.

Prediction of Conserved Kr-h1 Binding Sites on JHA-Induced Gene Promoters

After applying JHA for 12 hours, we extracted the sequences from the roughly 3 kb upstream untranslated region of the translation start site of the genes whose mRNA expression was increased at least 1.5-fold. The online MatInspector programme was used to examine these sequences for the conserved recognition and binding motif of transcription factor Kr-h1, a crucial JH signal transducer .

Silkworm Superlarvae Induction by JHA Application

We employed the JHA methoprene to generate silkworm superlarvae in accordance with other publications to assess the gene expression alterations in response to JH. We discovered that 10 or 20 g of JHA per larva effectively encouraged the development of silkworm superlarvae with a high probability of 93.3% when numerous doses of JHA were topically administered to recently moulted fourth instar larvae. A dosage of 30 g per larva, however, caused almost 50% of the larvae to die before their fourth moulting)use of JHA to induce silkworm superlarvae. Applying the JH analogue methoprene to freshly moulted silkworm larvae during the fourth instar resulted in the production of superlarvae. As a control, silkworm larvae were treated with just pure acetone. Effects of JHA treatment on survival and moulting of silkworm larvae. JHA treatment results in premature larval moulting and produces silkworm superlarvae at a dosage of 20 g per larva. A comparison of normal larva treated with pure acetone and normal larva that moulted prematurely 48 hours after JHA administration. The developmental time point occurs 60 to 72 hours following the end of the therapy. When JHA is applied, silkworm larvae's body weight changes. After JHA administration, Kr-h1, a JH-responsive gene, exhibits enhanced mRNA expression. hours after medication.

As a result, we fed hundreds of silkworm larvae 20 g of JHA each larva for more research. JHA administration caused an early fourth moult and an extra fifth moult, which resulted in superlarvae when compared to pure acetone treatment as the control and 1). The sixth larval instar of silkworm superlarvae caused the fourth and fifth larval instars to develop more quickly, but the entire larval stage was extended by about six days, and the average weight of each individual increased before wandering). The JH-regulated marker gene Kr-h1 also demonstrated a considerably elevated expression in the integument at 12, 24, 36, and 48 Hat) and in the fat body at 36 and 48 Hat, according to quantitative RT-PCR analysis of silkworm superlarvae.

JHA Application Caused a Broad Gene Expression Alteration

We employed a genome-wide silkworm microarray to examine the overall gene expression pattern in the integument after JHA administration using a model of JHA-induced silkworm superlarvae. 8,670 genes were expressed in the integuments with both JHA and pure acetone treatments, as shown in Table S2 and Figure S1. The treatment of JHA changed the expression levels of several genes, according to scatter plot analysis). It's interesting to note that following JHA treatment, 2,143 genes showed an expression change of at least 2.0-fold. For ease of use, we referred to these genes as JHA-modulated genes in the following description. 49 and 87 genes, respectively, underwent up- and down-regulation at 12 Hat. 69 and 74 genes, respectively, underwent up- and down-regulation at 24 Hat. 450 and 857 genes were upregulated and 165 and 956 genes were downregulated at 36 and 48 Hat, respectively. Additional real-time RT-PCR tests verified that majority of the studied genes, whose expressions were downregulated after JHA administration, displayed positive Pearson correlation coefficients between microarray data and RT-PCR findings .

JHA-modulated genes may be functionally categorised into 32 groups, according to GO annotation). The majority of JH-modulated genes were found to be associated with metabolic and catalytic processes. Additionally, KEGG analysis showed that the majority of the JHA-modulated genes were associated with metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems ; Table S5). Surprisingly, we discovered that JHA-modulated genes involved in fundamental metabolic processes had the highest number after removing redundancy for probes.

Most JHA-modulated genes in the integument showed expression changes that were similarly time course dependent, according to gene cluster-based coexpression analysis. The dynamic expression of JHA-modulated genes could be divided into eight groups, as shown in Figure 2 and Table S6. At 48 Hat, there was an upregulation of JHA-modulated genes from the C0, C1, C2, and C6 clusters. At 12 and 36 Hat, the C3 and C4 clusters were upregulated, whereas at 24 and 48 Hat, they were downregulated. At 36 Hat, C5 was upregulated, while at 48 Hat, C5 was downregulated. From 12 to 48 Hat, C7 shown a substantial downregulation. We utilised the MatInspector programme to look for transcription factor binding motifs in the upstream UTR regions of 157 genes whose expression had increased by at least 1.5-fold at 12 Hat after JHA administration. According to our findings, 95 of the 157 JHA-induced genes at 12 Hat included the conserved Kr-h1 binding motif, which is a crucial transcription factor for mediating JH signal . Importantly, as can be shown in Figure 2, all 67 JHA-induced genes' Kr-h1 binding motifs had the consensus sequence GGGT, which exhibited the greatest core similarity of 1 with Kr-h1's binding motif. This suggests that silkworm Kr-h1 may have these JHA-induced genes as direct targets[11], [12].

CONCLUSION

We further looked at JHA-modulated genes associated with metabolic pathways since they accounted for the biggest number of JHA-modulated genes and because JH's primary job is to support insect larval development. 99 JHA-modulated genes were found to be involved in carbohydrate metabolism according to our KEGG analysis findings, and 74 of these genes were found to be elevated in the integument following JHA application and could be grouped into the C0, C1, C2, C5, or C6 clusters. Intriguingly, we found that JHA-modulated genes involved in three different pathways of carbohydrate metabolism were all upregulated. These included ten genes for the pentose phosphate pathway such as ribose-phosphate pyrophosphokinase, 6-phosphogluconate dehydrogenase, 6-phosphogluconolactonase,

phosphoglucomutase, transketolase, fructose-1,6-bisphosphat Similar to this, JH application increased the expression of the majority of the genes associated with various other carbohydrate metabolism pathways, including glycolysis, pentose and glucuronate interconversions, galactose metabolism, starch and sucrose metabolism, amino sugar and nucleotide sugar metabolism, pyruvate metabolism, and glyoxylate and dicarboxylate metabolism.

In 14 pathways relevant to lipid metabolism, JHA regulated the expression of 58 enzyme-encoding genes (Figure 4; Table S8). 38 of these genes showed increased activity. After JHA induction, all 11 genes, including fatty acid synthase, 3-oxoacyl-[acyl-carrier protein] reductase, very-long-chain 3-oxoacyl-CoA reductase, stearoyl-CoA desaturase (delta-9 desaturase), and acyl-CoA oxidase, showed increased expression. Similarly, among 18 genes involved in the elongation and metabolism of fatty acids, 14 genes were upregulated after JHA application, including acetyl-CoA acyltransferase, palmitoyl-protein thioesterase, aldehyde dehydrogenase, long-chain acyl-CoA synthetase, very-long-chain acyl-CoA dehydrogenase, and enoyl-CoA hydratase. Four more genes involved in the production and breakdown of ketone substances were also activated by JHA.

After JHA administration, 34 of the 37 JHA-modulated genes involved in glycogen production and metabolism showed increased expression (Figure 4; Table S8). Notably, all JHA-modulated genes involved in the manufacture of different kinds of glycans (including N-glycan, mucin-type O-glycan, glycosaminoglycan, and lipopolysaccharide) had increased expression. In addition, JH-modulated genes for glycosaminoglycan degradation all increased their expression after JHA application, including alpha-1,2-mannosyltransferase, arylsulfatase B, hexosaminidase, xylosylprotein 4-beta-galactosyltransferase, mannosyl-oligosaccharide alpha-1,2-mannosidase, beta-galactosidase, and oligosaccharyltransferase complex subunit beta. 117 JHA-modulated genes were found to be engaged in 20 pathways relevant to amino acid metabolism, and 96 of these genes showed an upregulation after JHA administration, according to our KEGG analysis (Figure 5; Table S8). The metabolism of glycine, serine, and threonine, the degradation of valine, leucine, and isoleucine, the metabolism of arginine and proline, and the metabolism of tryptophan were among the JHA-modulated enzymes engaged in these pathways. For each of these four pathways, there were 23, 23, 19, and 17, respectively, JHA-modulated genes. Aldehyde dehydrogenase, 4-aminobutyrate aminotransferase, spermine synthase, beta-ureidopropionase, enoyl-CoA hydratase, and aldehyde dehydrogenase family 7 member A1 were just a few of the 12 enzyme-coding genes that were all upregulated after JHA application.

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CHAPTER 12
MANAGEMENT OF CLIMATIC FACTORS FOR SUCCESSFUL SILKWORM AND HIGHER SILK PRODUCTION

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ABSTRACT:
The genotypic expression in the form of phenotypic output of the silkworm crop, such as cocoon weight, shell weight, and cocoon shell ratio, is significantly influenced by seasonal fluctuations in the environmental components. The requirement for managing temperature and relative humidity for sustained cocoon formation is highlighted by fluctuations in the environment from day to day and season to season. The effect of temperature and humidity on silkworm growth and development, as well as new research on heat shock protein, are all covered in depth in the current review study. The impact of air and light on silkworm growth is also included in the study. In addition, this research focuses on how different environmental elements affect the development of a silkworm egg's embryo, the nutritional status of the larval stage, and the moth's capacity for reproduction. The research also emphasizes the need for caution during silkworm spinning and the impact of temperature and humidity on silkworm post-cocoon parameters. The research explored potential approaches for managing climatic conditions for a productive cocoon crop. 140 sources related to the issue are included in the study.

KEYWORDS:
Development, Environmental, Humidity, Silkworm, Temperature.

INTRODUCTION

The science of sericulture is concerned with the cultivation of silkworms for the manufacture of silk. Due to its glistening sheen, plushness, elegance, durability, and tensile qualities, silk is known as the "queen of textiles" and was first found in China between 2600 and 2700 BC. A natural fibre known as silk that comes from an insect's spittle is made from pupal nests or cocoons spun by silkworm larvae. Silk is favoured above all other kinds of fibres because of its exceptional qualities, including heat resistance, water absorption, dyeing effectiveness, and lustre. Temperature and humidity are the key environmental variables that affect an insect's physiology. Insects have a remarkable variety of adaptations to changing climatic circumstances despite the huge variations in their environment, and they manage to keep their internal temperature and water content within acceptable ranges. The process of adaptation is intricate and dynamic, and it greatly varies from one species to another. Insects' ability to adapt to changing environmental conditions relies on a number of factors, including dispersion, habitat choice, habitat alteration, association with water, cold tolerance, diapause, pace of development, sensitivity to environmental cues, and the synthesis of various cryoprotectant chemicals. Due to their extensive domestication over a period of 5000 years, mulberry silkworms are very fragile, extremely sensitive to environmental changes, and unable to endure severe natural temperature and humidity swings. As a result, the silkworm's capacity to adapt to environmental changes is quite different from that of wild silkworm and other insects. Depending on the mix of elements and developmental phases impacting growth, development, production, and quality of silk, temperature, humidity, air circulation,

gases, light, and other factors have a major interplay in their influence on the physiology of the silkworm[1], [2].

One of the most significant domesticated insects is the silkworm, which feeds on mulberry leaves as a larva to generate luxurious silk thread in the shape of a cocoon. Environment has a significant impact on silkworm growth and development. Ambient temperature, rearing seasons, the quality of the mulberry leaf, and the genetic make-up of the silkworm strains all have an impact on both biological and cocoon-related characteristics. Bombyx mori L. exhibits seasonal variation in behaviour. Seasonal variations in the environmental factors have a significant impact on how the genotype manifests itself in phenotypic traits as cocoon weight, shell weight, and cocoon shell ratio. The varying climatic circumstances over the last ten years have highlighted the need of managing temperature and relative humidity for long-term cocoon formation. India is comfortably ranked second in the world for silk production, behind only China. In India, sericulture has historically been practised in tropical climates like those found in Karnataka, Tamil Nadu, Andhra Pradesh, and West Bengal, as well as to a lesser degree in the temperate Jammu and Kashmir. The multivoltine and bivoltine hybrids are exceptionally tough and have a huge potential to live and reproduce under changeable ambient climatic circumstances, therefore the current tropical climate offers opportunity for their economic use. Ninety-five percent of the silk produced comes from multi-voltine hybrids.

The researchers assessed the multi-voltine silkworm's genetic potential and found possible parents for breeding projects. However, when compared to the current international standards, the multivoltine strain's silk quality is subpar. Over 80% of the world's silk is produced in China, with India being the second-largest producer with roughly 15%–16% of the overall output. China silk is of the highest calibre since it is of the bivoltine strain. Given these constraints, the introduction of bivoltine sericulture—which has the capacity to thrive even in the tropical climate of India—became urgent and impending. By using Japanese commercial hybrids as a breeding resource material, several prolific and qualitatively better bivoltine hybrids have been created in this line. However, the hot weather, especially in the summer, makes it difficult to grow these high-yielding bivoltine hybrids all year long. It is a well-known fact that, in contrast to polyvoltines, bivoltines are more susceptible to stresses in tropical environments. These stresses include hot tropical climates, poor leaf quality, and improper management of the silkworm crop during the summer, all of which make it difficult for farmers in India who are both technologically and economically underdeveloped to raise bivoltines . The impact of several environmental conditions on silkworm development, survival, production, and disease incidence is covered in this essay. The paper also reviews the results and conclusions of various researchers who investigated the effect of environmental factors on growth, development, feed conversion, reproductive potential, and postcocoon parameters of silkworms. The paper also discusses the ideal environmental conditions necessary for higher productivity in sericulture[3], [4].

Temperature Effects on Silkworm Growth

The development of the silkworms depends greatly on temperature. Since silkworms have cold blood, changes in temperature will directly affect their many physiological processes. The early instar larvae are often heat-resistant, which aids in enhancing survival rates and cocoon characteristics. The development of silkworms is directly correlated with the temperature; large temperature swings are detrimental to silkworm growth. Different physiological processes are boosted as the temperature rises, while they are lowered when the

temperature drops. The larval phase is shortened and growth is accelerated during silkworm rearing, especially in late instars. On the other side, growth is sluggish and the larval stage is extended at low temperatures. The ideal temperature range for silkworm growth is between 20°C and 28°C, while the preferred temperature range for optimal productivity is between 23°C and 28°C. The health of the worm is directly impacted by temperatures above 30°C. All physiological processes slow down around 20°C, particularly in the early instars. As a consequence, worms become too weak and more vulnerable to illnesses. The early instars have high temperature needs, and the worms actively eat, develop extremely aggressively, and have a fast growth rate. Later instars of such robust worms may endure more challenging environments[5], [6].

DISCUSSION

It is advisable to heat the room with electric heaters or charcoal fires during the winter and rainy season to manage the low room temperature. The greatest electrical heaters are those with thermo-regulators since they don't release any harmful gases. Charcoal that has been adequately cured may be employed when power becomes prohibitively expensive or unavailable in many rural regions of the sericulture belt. However, the carbon dioxide and other gases released during this combustion process harm silkworms and may be controlled by increasing ventilation, especially during the day. In addition, it's a good idea to keep the windows and doors closed, especially at night. Doors and windows should be opened in the evening as the temperature outside rises to let warm air into the space. All of the windows should be left open throughout the summer when the temperature is high during the day. On hot days, wet gunny cloth is placed over windows and doors to promote humidity while also lowering the temperature . Alternatives include using appropriate air coolers for this purpose. Numerous factors affect the sericulture industry's performance, but environmental considerations including biotic and abiotic factors are particularly crucial. Temperature is one of the main abiotic elements that affects silkworm development and production . There is a wealth of research indicating that excellent quality cocoons are generated in the 22–27°C range and that quality decreases beyond these temperatures[7], [8] .

However, polyvoltine breeds raised in tropical regions are known to adapt to the tropical climatic conditions and endure somewhat higher temperatures. It is essential to maintain a steady cocoon crop in a hot climate in order to deploy bivoltine races in a tropical nation like India. High temperatures have a negative impact on almost all biological functions, including the speed of biochemical and physiological responses, which may ultimately have an impact on the silkworm's ability to manufacture silk and the number or quality of cocoon harvests. The fourth and fifth phases were when silkworms were most susceptible to high temperatures, according to many studies. It is well known that the bulk of the commercially significant genetic characteristics of silkworms are qualitative in nature and that environmental variables like temperature, relative humidity, light, and feeding have a significant impact on phenotypic expression . The contributions of numerous variables, including environmental, racial, and other factors, on significant cocoon features are described findings show that reelability and cocoon output are the features most impacted by the unfavourable environmental variables. By lowering the temperature for 10 days, the first instar phase of silkworm larvae was lengthened Researchers also discovered that temperature and RH have an additive effect on the silkworm larval stage. These findings are consistent with those of previous researchers who found that changes in temperature and relative humidity had a significant impact on the moulting period Similar to this, other researchers' publications noted that a drop in temperature lengthens the time that silkworms spend

moulting. Table 2 shows how varied temperatures during late instars affect several silkworm cocoon features. Researchers found via a series of studies that tolerance to high temperatures is a heritable trait and that it may be feasible to develop silkworm races that are tolerant of high temperatures. The quantitative characteristics of silkworms in a recognised environment are of the biggest relevance in sericulture, according to many studies of various specialists . Researchers looked at how several traits of breeding line races were affected by low temperature during upbringing . In a similar vein, investigations of silkworm breeders discovered that low temperatures are always preferable to high temperatures in terms of silkworm production and larval duration for various instars. In contrast to their parents, the F1 bivoltine hybrids were shown to be more tolerant to high temperatures and high humidity .Researchers also observed that temperature and relative humidity were among the many variables that affect development, behaviour, and instar larval periods in silkworms and spoke about how insects can adapt to a variety of environmental situations in an important review. In-depth research has been done on how silkworm races adapt to environmental conditions, particularly temperature . Under subtropical and Kashmiri environments, the seasonal impact on silkworms was investigated . The impact of humidity on illness incidence and its impact on raising time was proven by several workers . For the warm and wet seasons in Uttarpradesh, India, suitable hardy bivoltine races developed and were put to the test in the field . Recent research on the effects of several seasons, including summer, rainy, and winter, on the cocoon and grainage properties of well-known bivoltine races in India came to the conclusion that temperature and humidity impact all of these races' characteristics . Nearly all biological processes, including the speed of biochemical and physiological responses, are impacted by high temperatures , which also has an impact on the amount and quality of cocoon harvests[9], [10].

Research on the Heat Shock Protein

In contrast to the exotic bivoltine races of temperate origin, the tropical Indian multivoltine races of Bombyx mori are more tolerant of high temperatures. Bivoltine races, in contrast to multivoltine races, have more production potential and produce silk of higher quality, but they cannot endure the harsh weather conditions seen in India. It is noteworthy to note that thermotolerance is usually accompanied by the presence of HSPs . It is known that when exposed to high temperatures, cells exhibit a heat shock response by synthesising a unique group of proteins termed heat shock protein . An accomplished scientist made an effort to comprehend the variations in thermotolerance between the multivoltine and bivoltine races. Also investigated was the heat shock reaction in mulberry silkworm races with varying thermotolerances. The necessity for thermotolerant bivoltine strains may be a crucial factor in creating bivoltine hybrids for tropical climates since several quantitative features diminish dramatically at higher temperatures. Recent developments in silkworm breeding and stress-induced protein synthesis have created new opportunities for the evolution of strong, fruitful silkworm hybrids . During thermal treatment, every genetic feature of the silkworms exhibited a decrease as the temperature rose over the norm. Similar findings were published by other researchers , who discovered that biological molecules including DNA, RNA, lipids, and other similar compounds were susceptible to heat stress. As a result of the usual pattern of protein synthesis ceasing, temperature stress results in a multitude of anomalies at the cellular level. However, it was shown that a short exposure of cells to a sublethal high temperature rendered the organism resistant to further and more severe temperature changes .To understand the genetic stability under diverse environmental circumstances and the productivity of different breeds under various environmental situations, it is thus crucial to

assess the degree of phenotypic variation of the economic features. It seems that years of thorough and intensive domestication prevented the insect from having the chance to develop thermotolerance. The major cause of the bivoltine strains' poor performance in tropical environments is temperature, among other considerations. When temperatures rise over 28°C, several quantitative characteristics do indeed suffer severe declines. Bivoltine breeds that are suited for the high temperatures and erratic climatic conditions of India are difficult to develop, according to researchers, particularly silkworm breeders in the sericulture area.

Humidity's Effect on Silkworm Growth

Humidity has a significant direct and indirect impact on silkworm rearing. The acceptable development of the silkworms and the creation of high-quality cocoons are significantly influenced by the interaction of temperature and humidity. It has a direct impact on the silkworm's physiological processes. The development of the worm is strong under situations of high humidity, which younger silkworms can tolerate better than older worms. Table 1 lists the ideal humidity levels needed for several early-age worms and late-age worms.

The pace at which the leaves in the beds used to raise silkworms wither is also indirectly influenced by humidity. The consumption of leaves by larvae will be reduced under dry circumstances, particularly in the winter and summer. This hinders the larvae's development and causes leaf waste in the rearing bed. Young larvae with retarded development are fragile and prone to illness. They can develop without being significantly hampered if the temperature is kept between 26°C and 28°C and the humidity is at least 90%. Similar to temperature, humidity varies greatly during the day as well as from season to season. The silkworm farmers must thus control it to ensure a good harvest. In order to increase humidity and prevent leaf drying during young-age raising, wax coated paper is used to cover the training beds. In addition, you may use wet foam rubber pads or water-soaked paper pads to raise the humidity in the raising beds. Rich farmers may control the humidity in the raising chamber using a humidistat-equipped humidifier. To promote consistent and effective moulting, it is crucial to reduce humidity to 70% or below throughout each instar's moulting period. Insect existence relies on their capacity to regulate and balance the water in their bodies, which makes up a substantial amount of their tissues. The majority of insects can develop at any humidity as long as they can maintain control over their water balance . There is no limiting range of humidity. The impact of high humidity on silkworm larval weight was investigated . Insects vary in water content from less than 50% to more than 90% of their entire body weight, and even when raised in comparable circumstances, there may be significant variance within a single species . The silkworm's development is influenced by environmental conditions, particularly the temperature and humidity at the time of rearing and the moisture content of mulberry leaves . Workers thoroughly researched how water and humidity affect sericulture . Research has also been done on the impact of the seasons and humidity on silkworm development and nutritional effectiveness . Researchers discovered a race that is well suited to spring and fall raising . Numerous researchers have investigated the impact of humidity on various seed production factors . Additionally, the phenotypic and genotypic traits for each season were documented by researchers . Additionally explored were the behaviours of polyvoltine races in the arid climate of the Rayala seema regions of Andhra Pradesh, India . Humidity is an abiotic element that significantly affects how well insects operate in terrestrial habitats. Humidity influences growth and development mostly via indirect means by interacting with the availability of free water and the water content of the diet. Humidity requirements change based on the biological cycle. The impact of unfavourable environmental circumstances on the successful production of bivoltine cocoons

was investigated . Researchers in Italy looked at how different environmental conditions affected the development of silkworm larva . Seasonal variations, atmospheric humidity, and soil moisture percentage all have a significant impact on the development and quality of mulberry leaves, which in turn affect silkworm health and cocoon crop production. This suggests the significance of leaf moisture for both flavour and absorption of the leaf's nutritional components.

The Effects of Air and Light on Silkworm Growth

Silkworms need access to fresh air much like other creatures. Carbon dioxide gas is released in the raising bed by the breathing of silkworms. The amount of CO2 in the air may be used to gauge its freshness. Although the air CO2 level in the raising chamber is typically between 0.03 and 0.04 percent, when farmers burn charcoal to boost warmth, other gases like as carbon monoxide, ammonia, sulphur dioxide, and others are also emitted. Because these gases are harmful to silkworms, care should be taken to ensure that fresh air is let in via adequate ventilation in order to maintain low levels of the poisonous gases. Silkworm development is slowed down if CO2 concentrations are higher than 2%. Additionally, disinfectants and insecticides are not allowed in the raising chamber. Young silkworm larvae are particularly sensitive to toxic gases, thus artificial air circulation is very helpful in reducing the level of polluted air. In comparison to data acquired under conditions of zero ventilation, the air current of 1.0 m/sec during 5th-age rearing decreases larvae mortality and enhances intake, digestibility, larval weight, cocoon weight, and pupation rate .

Due of their photosensitivity, silkworms have a propensity to crawl towards the direction of low light. They dislike both intense light and total darkness. Silkworms that are raised under constant light have a growth delay. Additionally, it results in pentamoulters and lowers the weights of both larvae and cocoons. Silkworms like 15 to 20 lux of low light and stay away from complete darkness and intense light. In cycles of 16 hours of light and 8 hours of darkness, late-age worms do better. However, immature worms like a 16-hour darkness phase and an 8-hour period of light. Strong light or total darkness are not preferred by silkworm larvae, although often, the light phase, as opposed to the dark phase, awakens the larvae. The silkworm is a short-lived insect with positive phototactism . The larvae are fed in full darkness throughout the life cycle, which prolongs their larval stage and lowers the quality of their cocoons . Growing and moulting are erratic when babies are raised in either total darkness or intense light. Larval duration is often longer during the light phase than during the dark phase. A thorough investigation was done into how light and temperature affect silkworm development .

Environmental Aspects' Effects on Embryonic Development

There has been substantial research on how temperature affects silkworm growth and development, but less focus has been placed on how temperature affects embryonic development. According to one study, when the rate of development is plotted against temperature in exothermic species, a sigmoidal curve is formed with an approximately linear connection in the middle temperature range. Temperature is a developmental cycle parameter that may be experimentally altered, although the results are highly difficult to understand. Embryonic mortality after exposure to fatal temperatures is thought to have a very complicated physiological cause that is likely species-specific. Throughout the hatching and raising stage, improper egg incubation causes a number of issues. When a silkworm egg is incubated at a high temperature with low humidity, the larvae's ability to hatch is severely hampered. It is widely known that the environment during embryonic development influences

not only the length of the larval/pupal stage, the weight of the cocoon, and egg production . The egg stage of the Bombyx mori silkworm has the lowest resistance to high temperatures of any developmental stage. Given that the embryonic stage is the most susceptible to temperature, incubation temperature has an impact on voltinism character as well . While bivoltine eggs incubated at lower temperatures result in moths that lay mixed and totally nonhibernating eggs, those incubated at higher temperatures yield moths that lay hibernating eggs. Temperature has a direct impact on development rate, and humidity has an impact as well. When the temperature is high, the embryo develops more quickly until the stage of setae creation, at which point it dies because the yolk cannot keep up with the rapid rate of development and obstructs proper growth . According to Kittlans , temperatures higher than 33°C and unusually cold handling of embryos may also result in embryonic mortality or aberrant development. Tetraploid silkworm people are created when the eggs are exposed to cold, and they deposit enormous eggs .

For a proper development of the embryo during silkworm egg incubation, humidity must be maintained at 80% on average. Low hatching rates are a given if humidity levels during incubation fall below 70%. The water content of the insect is one of several critical elements that complicates how humidity affects respiration. The effects of humidity are strongly correlated with temperature, including desiccation-related water loss, spiracular diffusion, ingested water retention, and metabolic water synthesis . Silkworm eggs lose water when the relative humidity is below 60%, and when it is over 90%, physiological waste water is retained within the egg, harming the developing foetus. For the bivoltine silkworm, the impact of light on embryo incubation and development, the need of black boxing at the pinhead stage, and exposure to light on days 10 or 11 were all carefully examined . Temperature and humidity together have a significant impact on the physiology of the egg . However, for both bivoltine and multivoltine eggs, the optimal temperature ranges from 25°C to 75–80% relative humidity . In-depth research was done on the prevalence of unfertilized eggs in silkworms and its cause . Researchers investigated the impact of chilling on hatching and rearing characteristics in multivoltine eggs during the blue stage .

Environmental influences on nutritional indices

Insects can only operate to their physiological capacity when put in an optimal and favourable setting; variation in temperature variations inhibits this. Insects have developed specific skills to assess their surroundings and make choices including physiological, behavioural, and genetic responses as a result of natural selection driven by less optimal environmental circumstances. Changes in food intake and utilisation, feeding frequency and timing, metabolism, enzyme production, nutrient storage, and other physiological and behavioural processes are typical components of these responses. Abiotic elements that are highly variable in natural surroundings have an impact on how food is consumed and used. If environmental changes prevent insects from performing as well as they would under ideal circumstances, their performance may suffer unless their physiology and behaviour are altered to adjust. Performance may be affected differently by variable temperature regimes compared to constant temperature; growing performance is often encouraged under variable temperature regimes . Additionally, insects have developed a variety of metabolic and enzymatic adaptations that enable them to live and grow in a wide range of temperatures. Individual insects can adapt to varying degrees of changes in the ambient temperature thanks to temperature acclimatisation, physiological thermoregulation, and behavioural thermoregulation . The buildup of organic matter that results from the equilibrium between anabolic and catabolic processes, which are fueled by the nutritive ingredients taken after

meal digestion, is how silkworm development is visible. When fed on leaves of various nutritional qualities, silkworms of the same genetic stock reacted in various ways, which is a sign of effective food utilisation and conversion into silk material. When temperatures rise beyond 30°C, metabolic processes become irregular and have a negative impact on health. When temperatures drop below 20°C, metabolic processes also become dormant once again, causing uneven growth and declining health .

There have been reports of variations in ingesta and digesta levels across breeds as well as within the same breed throughout various seasons . On larval development, weight, and survival, the rate of food intake and leaf quality were evaluated .However, efficiency metrics like ECI cocoon were greater for batches of silkworms kept at higher temperatures, which may be related to fewer feed alternatives, which result in some physiological adaptations to combat nutritional stress[11], [12].

CONCLUSION

Understanding the racial variations in the digestive and assimilation capacities of the silkworm will benefit from analysis of the nutritional indicators, such as the rates of intake, digestion, assimilation, and conversion in the developing larvae. Based on food utilisation efficiency at various feeding quantities under ideal circumstances for each sex, strains must be evaluated . According to researchers , the rate of leaf-silk conversion diminishes as temperature rises . The silkworm's body temperature affects its physiological processes, food intake, and economic factors. There was a correlation between an increase in mulberry leaf consumption and a drop in rearing temperature, according to one study . Researchers looked at how different environmental conditions affected the nutritional and hydration needs of insects . According to research on the impact of temperature on silkworm leaf-silk conversion, low temperatures throughout the rearing stage encouraged increased silk conversion and improved survival in bivoltine silkworm . In larvae raised at low temperatures, a high conversion efficiency was seen . Under various climatic circumstances, researchers examined the nutritional indices and nutritional efficiency characteristics of popular India bivoltine race 5th instar larvae . They showed that under ideal temperature and humidity circumstances, nutritional indices metrics as ingesta, digesta, approximate digestibility, and reference ratio were better. The impact of leaf moisture on nutritional parameters and the development of the silk gland in silkworms was investigated by many authors.

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