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Indigenous knowledge of silkworm cultivation and its utilization in North Eastern region of India

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The vegetation of Northeastern region is unique being characterized as one of the richest flora in the world, which produces a variety of products. Northeastern India has the highest number of endemic plants, animal and microbial species. Many sericigenous insects along with their food plants are endemic to this region. Sericulture and weaving are part of the cultural heritage of the Northeastern region and is one of the most promising income sources to this region without spending much for its cultivation.

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Sericulture is an agro-based industry, playing an important role in rural economy of the country. The uniquely golden coloured *muga* silk has restricted distribution and is found only in the NE part of India. Assam is the only state in the country producing all the four varieties of silk. *Eri* silk, also known as *errandi* or *endi* silk is produced by *Philosomia ricini*, which is the only completely domesticated non-mulberry silkworm. *Tasar* silkworm, (*Antheraea mylitta*) rearing is another important sericultural activity in this region of the country. Prevention of silkworm diseases and breeding of a silkworm variety with high productivity are important problems in the commercial aspects of sericulture.

Traditional practices associated with silkworm rearing

Sericulture and silk weaving is the part and parcel of cultural heritage of the people of NE India. The climate of North -east India is suitable for growth of non -mulberry silkworms, i.e. *muga* and *eri*. The number of sericulture village in NE region is about 38,000 and approximately 1.9 lakh families are engaged in this industry in Assam. Geographic Indication Right has been conferred to this stunning *muga* silk yarn, its dazzling fabric and the other products based on these two.

Muga rearers of Assam have been practicing this culture with traditional methods. The rearers, throughout the rearing processes are following a large number of indigenous practices. Some of these practices are having scientific backgrounds while some of them are found to be superstitious. Indigenous technologies developed as a result of age-old practices of sericulture in the region can be grouped into categories like pre-rearing technologies, during rearing technologies, etc. Rearers collect eggs from other source or prepare eggs from the moths emerging out from a selected portion of cocoons from the previous crop following some traditionally approved criteria for the cocoons. Since, there were no such morphological characters distinguishing one brood of worms to other, rearers select seed cocoons with utmost care based on size of the cocoons, habit of the larvae, response to physical touch, eating behaviour, etc. This imparts a selection pressure on the *muga* populations for getting the best cocoons for getting best broods of eggs and worms out of it[1]. *Muga* sericulture, being a semi-wild rearing practice needs host plants for a major part of the life cycle of the insects. To protect host plants like *Som, Sualu,* etc. from grazing animals, *muga* rearers traditionally apply fresh cow dung on the base of the plants. Controlled burning of undergrowths in the host plantation prior to rearing is another practice followed by the farmers, which helps in removal of pest and

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predator infestations, disease causing microflora, etc. Another cost effective disinfection practice is being followed by the traditional rearers is to hang all the equipments used in rearing above their kitchen fire. This practice is effective as continuous exposure to high temperature helps in desiccating pebbrine spores and other disease causing microbes from the equipments[2]. To incubate the eggs for hatching, rearers use banana leaves to spread the eggs which is a traditional technique backed by the scientific judgment of availability of appropriate temperature and humidity and protection of newly hatched larvae from injury and desiccation. During rearing, worms are brushed by using *Khorika* made of thatch grass to the base of plant. This practice is reported to be very much useful in many ways like avoidance of frequent handling, reduction of required number of man days for transferring the worms and also in putting some natural selection pressure on the worms[3]. *Muga* rearers of the state are found to restrict the entry of outsiders to the fields during the rearing period. The rearers also do not move out of the field. Both these practices are found to be sanitary measures against secondary contamination of silkworm diseases. *Muga* rearers of the region take the entire activity of rearing as one holy practice and hence follow many moral and ethical practices in various phases. Although these practices are sometimes without scientific background, strict adherence to these roles ensures a certain amount of dedication on the part of the rearers and this ensures a better crop from the practice[1].

Potential areas of application of molecular biology and biotechnology

The recent advances in molecular biology and biotechnology could play a major role for improvements in the sericulture sector. Use of biotechnology in improving the productivity of silk, the quality of yarn and qualitative and quantitative improvement of the host plants, etc. are some of the potential areas in this regard. Another problem related to sericulture is the sharp decline in biodiversity and population density of wild silk moths. India with its diverse environmental conditions is known for the local races of silkworms that are rich reservoirs of many resistant genes. These genetic resources can be used for development of disease resistant hybrids in sericulture and molecular markers as a tool can be used to study the inheritance of such complex traits. Study of the bio-ecology of wild silkworm is also needed to protect these genetic resources and their ecologically diverse habitats[4].

The use of molecular markers is well suited to the genetic improvement of the silkworm [5, 6]. PCR based molecular marker, random amplified polymorphic DNA (RAPD) technique was used to study the DNA profiles of *muga* and *eri* silkworm strains. Eleven *muga* silkworm strains were analysed using 50 random primers among which 36 polymorphic primers gave 309 amplicons. The average amplicons per primer was found to be 8.58 and 94.82% amplicons were polymorphic. Cluster analysis based on Jaccard's similarity coefficients resulted in the formation of two main clusters with S9 on one cluster and the remaining populations on the other cluster. Jaccard's similarity coefficients ranged from 0.122 to 0.863 indicating a high level of diversity within *muga* silkworm collection. Four strains of *eri* silkworm (Fig. 1) were characterized based on their protein profile by gel electrophoresis (SDS-PAGE) and DNA by RAPD technique. Eight random primers and one universal primer used for amplification generated a total of 31 bands of which 25 (80.64%) were polymorphic. In both SDS-PAGE and RAPD, the UPGMA based dendrogram showed two clusters. The range of genetic diversity observed among the strains of both *muga* and *eri* silkworms affirms the potentiality of RAPD technique for identification and selection of distant parents for silkworm hybridization for high silk yield [7].

Application of tissue culture technique

An *in vitro* approach to study the brain of silkworms has been successfully tried. The brain of the fifth instar second day larvae of silkworm is the capable form to survive *in vitro* for about 14 to 16 hrs at 37°C in the dark. The brain media were screened for microbial growth, filtered and passed through 10 Kda and 30Kda, molecular cut off filter units (Millipore, USA) and then through Sephadex G-10 (desalting column), exclusion volume collected, lyophilized and subjected to SDS, NON-SDS and other molecular separation techniques. The molecular weight of this protein/peptide is found to be in between 40 Kda – 23Kda[8,9]**.** RNA was isolated from the brain of the silkworms and the RT-PCR studies showed the presence of Allatostatin type neuropeptide in the brain[18]. The linkage between the neurosecretory cells, neurosecretory materials, peptides and proteins with respect to juvenile hormone biosynthesis have also been demonstrated. Further, its effect on silk biosynthesis within the silk gland was demonstrated with the help of synthetic juvenile hormones and its analogues in particular silkworms. The application

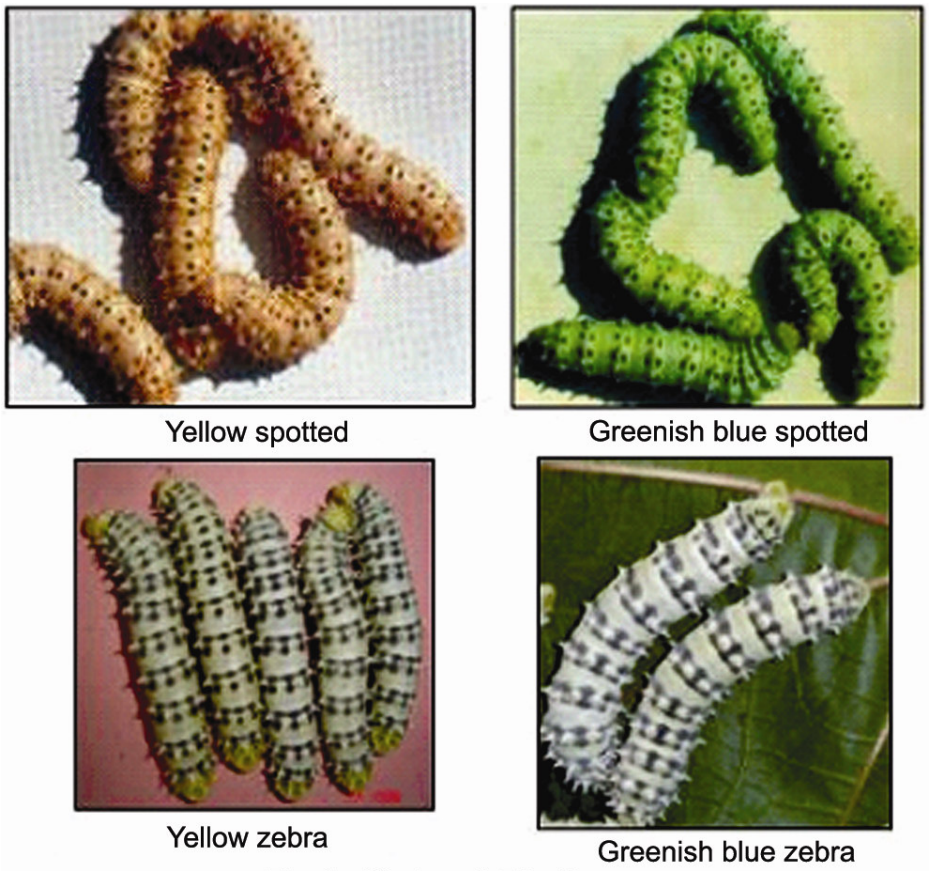

Fig. 1 - Strains of *Eri* silkworm

studies of these factors on silkworms in laboratory and field trials caused a significant response on the silkworms in terms of better larval growth, silk gland weight and yield of silk fibre from 3-6%. The progress has already generated interest in North Eastern region, which can be utilized in sericulture for the better silk yield in terms of quality and quantity.

Host plant improvement

Food plants play a major role in sericulture because the leaves are the only dietary source converted into silk of high commercial value. The three major factors viz, survival rate, silk production in terms of quality and quantity and fecundity depends on the selection of nutritive superior plant values. Propagation and improvement of host plants are equally important for increasing the *muga* silk yield. *In vitro* culture of tissues, cells and protoplasts have become useful tool for crop improvement through genetic manipulation as well as propagation. Establishment of germplasm bank is the prime prerequisite to use them for improvement of the host plants through breeding. For this purpose exploration, collaboration, maintenance and evaluation of diverse gene pool are required.

Plant growth promoting rhizobacteria (PGPR) were isolated from the vicinity of *Som* plants (*Machilus bombycina*) and screened for effectiveness by spraying combinations of strains on the *Som* plants. The effects of the PGPR combinations through increase in chlorophyll content, free amino acid, total protein, reducing sugar, carbohydrate and dry weight were studied. The strains showing growth promoting activity in combinations of RB1 + RB3 + RB4 + RB5 + RB8 strains produced the best result. *Muga* silkworm larvae fed on the *Som* leaves of the plant treated with this strain combination had higher activity of the enzymes, viz. Trehalase, transaminase and phosphorylase in the silk gland, haemolymph and fat body. The cocoons of these silkworms produced more silk in terms of quality and quantity. This study could be exploited for improvement in quality and quantity of silk production through the application of PGPR[10].

Bio-control of silkworm disease

The flacherie disease is causing considerable damage to the silkworm, especially *muga*, *Antheraea assama*. Other than preventive measures, no remedial

measures have so far been developed to check the infection and further spread of disease. Of the different bacterial strains isolated from diseased *muga* silkworm, the strain named as AC-3 was found to be most pathogenic to the silkworm. The induction of antibacterial proteins in haemolymph of silkworm can be achieved by injecting live non-pathogenic strain of *Pseudomonas* DAS-01. Protein profile of control and disease induced pupa were compared. In disease induced pupa 3 protein/peptide bands were found in low molecular weight region (18-24 kDa). The fraction containing low molecular weight proteins were found to be effective in inhibiting the growth of *Pseudomonas aeruginosa* AC-3. The second fraction collected after further purification of these induced protein by HPLC showed maximum antibacterial activity when tested against *Pseudomonas* AC-3 strain[11]. The survival percentage was higher in immunized set of larvae as compared with their respective controls. The molecular weight was determined by LC-MS and was found to be positively charged with molecular weight 23 kDa and further analysis of this purified protein by Aminoacid analyzer showed the presence Glycine, Lysine, Leucine, Isoleucine, Alanine, Arginine,Valine, Glutamic acid, Proline and Aspartic acid[12-15].

A successful attempt was also made to control the flacherie by extract of some medicinal plants occurring in the NE India. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Different antibiotics and plant extracts were tested for their effectiveness in inhibiting the growth of AC-3. (*Helica*) *Terminalia chebula* extract was also found to be most effective against the strain[16]. The stability of extract has also been studied and tested at the farmer's level. The combination of *Helica* and Gallic acid (one of the constituent of *Helica*) enhanced the survivability of larvae from 30-75% and silk production from 15-20%, respectively. The product has been very recently released to progressive *muga* farmers[17].

NEIST-Jorhat has developed a novel method for improved cocooning of *muga* silkworm *Antheraea assama* Ww. The method comprises identifying a tree which is known to naturally host *muga* silkworms, cleaning the proximal area around the base of the trunk of the said tree trunk with a top open cage like structure containing clean and dry straw, allowing natural maturing of the said *muga* worms on the said tree followed by entrapment of the straw contained in the said cage, further allowing the straw entrapped mature *muga* worms to spin cocoons in the said filled cage, and harvesting the fully spun cocoons by physically separating from the straw[18].

Conclusion

Muga sericulture hold the tremendous scope for development of rural economy of the region as it has the advantages of close association with the tradition and culture of local populace, availability of abundant nature grown host plants, eco-friendly production process and skilled household in rearing, reeling and weaving. So, utilization of the available technological know-how and development of better technologies is the call of the hour in this sector.

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