Rain Forest Research Institute, Jorhat, Assam

(Indian Council of Forestry Research & Education)

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Submitted by-

Archita Dev Barman

Vellore Institute of Technology, Vellore

B. Tech, Biotechnology

School of Biosciences and Technology

<u>Project Title</u>: **Initiation of in vitro cultures of high yielding morphotypes of *Persea bombycina* King.**

1. **<u>Aim</u>**: *In vitro* propagation of som plant (*Persea bombycina* King)
2. **<u>Introduction</u>**:

Only India is capable of creating the gleaming golden Muga silk. It is primarily produced in Assam and a small number of other North-eastern states. *Antheraea assama*, a Muga silkworm, produces Muga silk. *Persea bombycina* (King ex Hook. f.) Kost. (Som, local name) and *Litsea monopetala* (Roxb.) Pers. (Soalu, local name) of the Lauraceae family are the main hosts for Muga silkworm. In North Eastern India, they flourish, especially in the Garo Hills and Brahmaputra Valley. Som trees often grow more frequently in upper Assam and generate reeling cocoons, whilst Soalu trees typically grow more frequently in lower Assam and create seed cocoons. Muga silk has a huge market potential on both the domestic and global levels. The culture of this priceless silkworm is practiced by a significant portion of the population in northeastern India.

Since seeds used to propagate som typically have substantial genetic variability, commercial plantations find them less desirable. To increase the production of Muga silk, the Central Muga Eri Research and Training Institute (CMER&TI) has discovered a few superior morphotypes of the Som host plant. The Muga silk industry will grow as a result of the micropropagation of these Som, which will increase production and productivity. For the commercial cultivation of Muga silk, a considerable number of Som plants with exceptional morphotypes are required. Micropropagation is a faster form of plant reproduction than normal vegetative propagation. Tissue culture makes it possible to grow considerably more plants, necessitating the need for a Som micropropagation methodology.

The nutritional value of the leaves fed has a significant impact on the growth and development of silkworm larvae as well as the economic characteristics of cocoons. According to Matsumara et al. (1958), among the several characteristics that contributed to the performance of the crop, the leaf ranked first (38.2%), then the climate (37%) and rearing practices (9.30%), followed by silkworm race (4.02%), silkworm eggs (3.10%), and other factors (9.60%), etc. The host plant's leaf protein serves as the direct source of over 70% of the protein in silk generated by the silkworm. The nutritional value and seasonal variation of the host plants are quite similar to

that of the silk worm. Therefore, one of the crucial elements in sericulture is selecting a host plant that will support the proper development of silkworms.

The silkworm's preferred primary feeding plants are Som (P. bombycina) and Soalu (Litsea monopetela). Upper Assam's (Dibrugarh, Sibsagar, Lakhimpur, Jorhat, and Golaghat districts) and lower Assam's (Darrang, Kamrup, Karbi Anglong, and Goalpara districts) vast plantations are both naturally rich in som, which is economically exploited for the production of reeling cocoons. The Som leaf's nutritional content has a significant impact on the growth and development of silkworms. The likelihood of getting high-quality cocoons increases with the quality of the host plant's leaves. Similar to how silkworm growth, the quality of the cocoon, and the quantity of raw silk are all entirely dependent on the quality of the leaves. Low productivity is mostly caused by diseases, unfavorable weather, insect pests, inadequate agronomic techniques, and undesirable weeds. Numerous foliar diseases that impair Som's normal growth, the number, quality, and eventual generation of cocoons are susceptible to the plant.

In Assam, there are over 38,000 villages dedicated to sericulture, and 1.9 lakh families work in this field. Unni et al. (2009) claim that Assam is the only state in the nation to produce all types of silk. Districts in Upper Assam and a few locations in Lower Assam practice the Muga silk culture. The Goalpara district in Lower Assam produces 800 lakhs of Muga cocoon per year.

P. bombycina and L. monopetala, the main hosts of the Muga silkworm, are found across northeastern India, but are particularly abundant in the Brahmaputra River and Garo hills. The Brahmaputra valley has som up to an elevation of roughly 500 metres. It is sparsely dispersed throughout Assam and extends to the lower Himalaya, Meghalaya, the Khasi and Jaintia Hills, and Nepal's western border. However, no outside of India research on these plants has been documented.

3. **Methodology**:

3.1 **Collection of explants**:

The explants (S3) were collected from the mother plant from the field of RFRI, Jorhat. Actively growing lateral branches which are 30- 60 days old were utilized as source of explants. These branches were collected using sharp secateurs. Lateral branches were then brought to laboratory for further processing.

3.2 **Preparation of explants**:

Since emphasis was given for micropropagation from mature explants, special attention was required for their surface sterilization to reduce the endogenous contamination. The leaves, tender shoot tips and overmatured shoots were discarded while keeping the matured shoots safe. After removing the leaves, the whole branches were wiped with cotton swab dipped in 70% Ethanol. The leaf sheaths were then removed using a sharp scalpel without causing any damage to the dormant bud. After that, the nodal explants were prepared by making 3-4 cm long pieces each containing a single node. The nodal explants were put in a beaker containing 100 ml sterile water and then added the fungicides (0.5 gm Indofil + 0.5 gm Bavistin). 1 drop Hi-clean liquid detergent is also added to the solution. It was kept for 30 minutes while being periodically shaken. It was then washed multiple solution until it became foam free. After that, we have prepared a 0.5% ascorbic acid solution and then added to the beaker containing the explants. It was also shaken periodically for 2 hours. It was again washed for multiple times in order to make it prepare for the actual surface sterilization.

3.3 **Surface sterilization of explants**:

The explants were then taken to laminar air flow cabinet for the actual surface sterilization of the explants and to study the effect of different sterilization procedures. All the things inside the LAF such as media bottles, spirit lamp, forceps stand and holder, culture plates, etc. were sterilized using 70% ethanol. We have followed two sterilization procedures:

(i) Under the LAF cabinet, the prepared explants were washed with 0.1% $HgCl_2$ and 1% (V/V) solution of Tween 20 (2-3 drops) by shaking for 5 minutes in a bottle. Then it was rinsed with sterile distilled water for 4-5 minutes. After that, inoculation was performed in the culture media (semi-solid).

(ii) In the second procedure, 1% (V/V) solution of Tween 20 (2-3 drops) was added to the bottle containing explants outside the LAF. It was kept for 30 minutes while being periodically shaken. Before taking it to LAF, it was washed for 3-4 times with sterile water. Then, inside the LAF cabinet 0.1% of $HgCl_2$ was added which was shaken in a bottle for 5-7 minutes. Again, it was washed with sterile water.

3.4 **Culture condition**:

The culture tubes and bottles were kept in growth room and allowed to grow in controlled environment. The temperature of the growth room was maintained within 25±2°C by air conditioner. A 16-hour light period was maintained by photoperiodic timer with light intensity of 2000 Lux for the growth and development of culture.

3.5 **Preparation of stock solution**:

Since weighing the essential elements every time a medium is made is a time-consuming and laborious operation, concentrated solutions with the desired medium composition are made and then utilized to make media via proper dilution. Stock solutions are the name given to these concentrated solutions.

Macronutrient stock solution was made at a 10X concentration. With the exception of CaCl2, each macronutrient was dispersed individually. In order to prevent precipitation, CaCl2 was first dissolved separately and then added to the remaining stock solution. All of the micronutrients were produced as a 1000X concentration stock solution.

Stock solution of vitamins was prepared in 100X concentration by dissolving them in distilled water. Stock solution of cytokinin and auxin were prepared at 1 mg/ml concentration by dissolving in required solvent. Stock solutions of auxins (IAA, IBA and NAA) were prepared by dissolving them in minimal volume of ethanol or 0.1N NaOH as a solvent and made up the volume with distilled water. Stock solutions of cytokinin were prepared by dissolving them in minimal volume of 0.1N HCl as a solvent and made up the volume with distilled water. Stock solutions of kinetin was prepared by dissolving 0.025 gm in 1N NaOH.

3.6 **Preparation of medium**:

We have prepared both hormone and hormone free media with varied compositions:

(i) MS + Hormone free

(ii) MS + BAP (3mg/L) + GA3 (0.5mg/L) + Kinetin (0.5mg/L)

(iii) MS + BAP (1.5mg/L) + Kinetin (0.5mg/L)

To prepare 1 liter of nutrient media required volume of stock solutions of nutrients and plant growth regulators were added sequentially in 200 ml distilled water. After that sucrose (30

gm/ L) was added. Finally, the volume is made up with addition of distilled water. The pH of all the media were adjusted to 6 in general by adding 1N HCl or 1 N NaOH except the media which were used to study the effect of different pH. Sterilization of media was done by Autoclaving at 15 psi / 121^0 C for 15-minutes.

The autoclaved media thus prepared were kept for 3 to 4 days prior to use to see if there is any microbial growth.

4. **Observation**:

Out of the two sterilization procedures performed, treatment 1 where the explants were washed with 0.1% HgCl2 and 1% (V/V) solution of Tween 20 (2-3 drops) inside LAF showed maximum contamination after inoculation for almost two weeks. Only 3-4 cultures out of 20 survived while rest were contaminated by bacteria and fungi. While the second sterilization procedure showed comparatively less contamination of cultures where 8 cultures survived out of 20.

Fig 1: Mother plant (*Persea bombycina* King) from RFRI, Jorhat

Fig 2: Leaves of Som plant (*Persea bombycina* King)

Fig 3: Plate containing nodal explants inside LAF

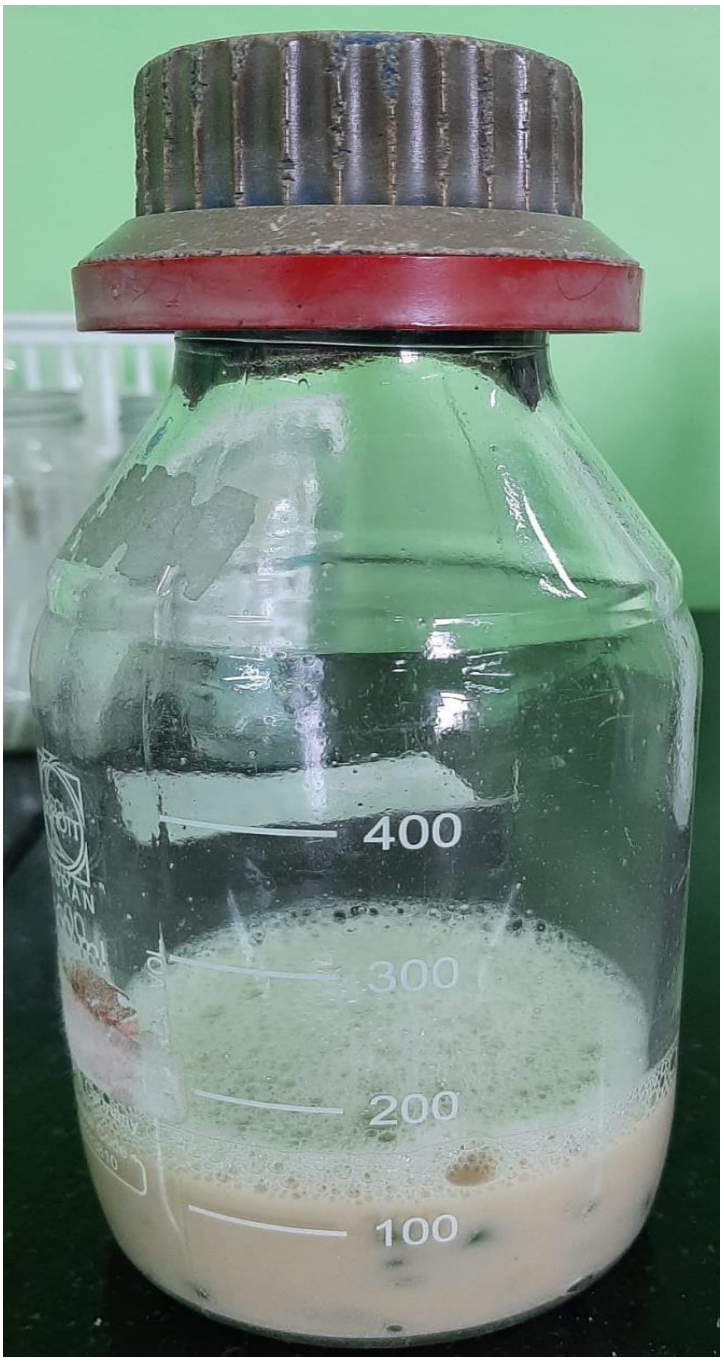

Fig 4: Explants washed with Fungicide (Indofil + Bavistin)

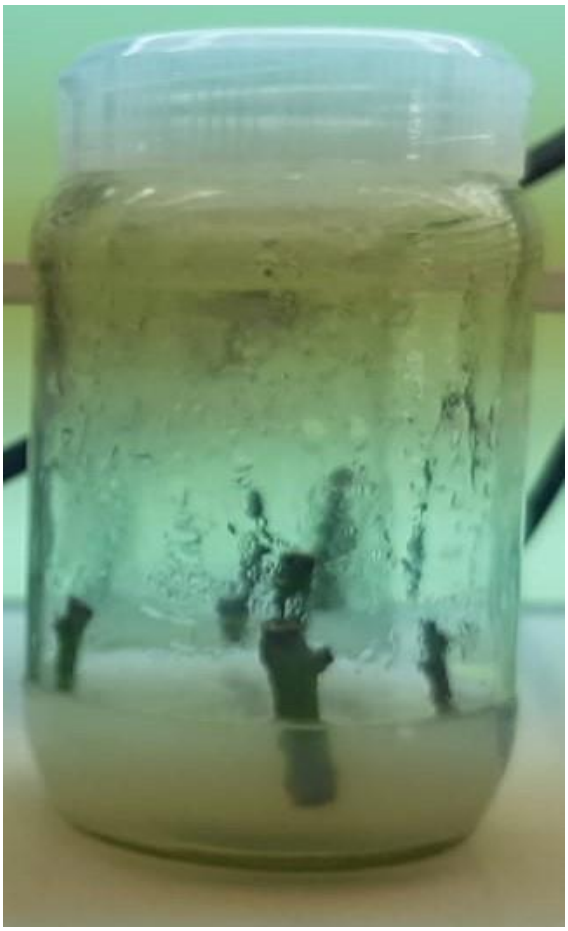

Fig 5: A nodal explant in semi-solid MS medium just after inoculation

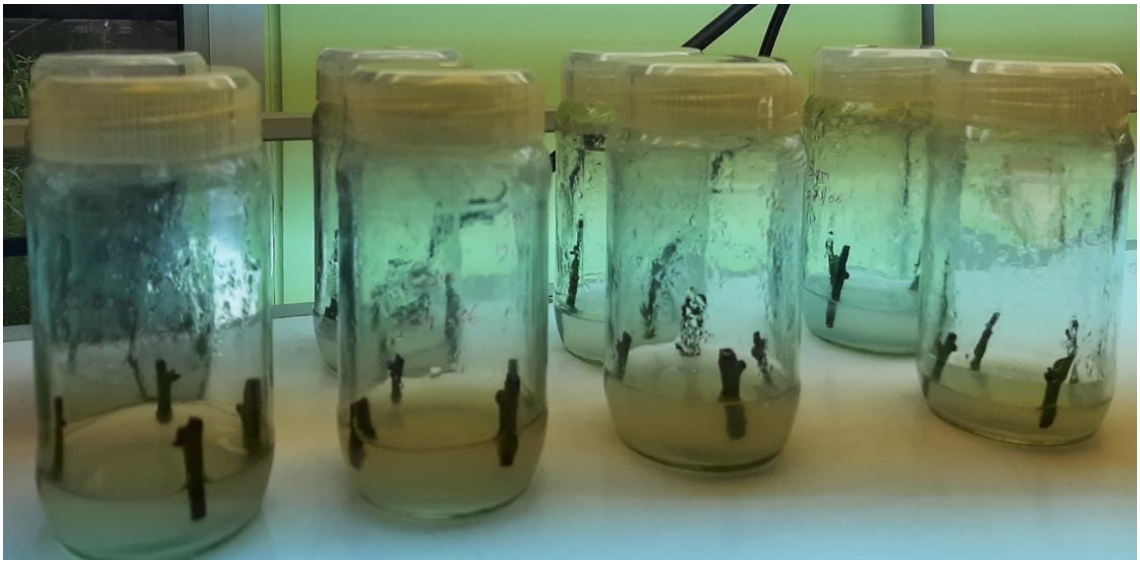

Fig 6: Contamination free explants after 2 weeks of inoculation

Fig 7: Nodal explant contaminated with fungi

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