Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 04, Issue: 03 (MAY-JUNE, 2024)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Sericultural Grainage Techniques in India

(*Kishore S. M.[1] and Bharthisha S.M.[2])
[1]Ph.D. Scholar, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga
[2]M.Sc. Scholar, Agronomy, University of Agricultural Sciences, Dharwad
*Corresponding Author's email: kp464751@gmail.com

Grainage, derived from the French word "graine" meaning "seed," pertains to the meticulous establishment of robust silkworm eggs for optimal production. Employing a systematic approach not only mitigates mortality rates and reduces labor requirements but also enhances the quality of offspring. The selection and preservation of seed cocoons, the preparation of disease-free layings and their disinfection and incubation are among the important aspects of grainage. Grainages are the places where the disease-free layings (DFLs) of silkworm called industrial seeds are produced on a mass scale to be supplied to commercial rearers. The industrial seeds supplied are generally hybrids. The hybrids may be a simple cross of two multivoltine races or a Cross Breed of a local multivoltine with a bivoltine or a polyhybrid cross of more than two races. The hybrids by virtue of their hybrid vigour or heterosis are superior to the parental races.

For profitable sericulture industry two things are important:

1.The race of silkworm reared should be superior and spin commercially good quality cocoons. 2. Healthy and hygienic rearing must be carried out by providing optimum conditions and quality leaves.

Exotic Chinese and Japanese silkworm strains, renowned for their superior cocoon quality, struggle to thrive under the prevalent conditions in India, making mass-scale commercial rearing impractical. Consequently, hybrid silkworms are bred, combining the resilience of local strains with the high yield of foreign breeds, to meet commercial demands effectively.

To streamline the production of high-quality, disease-free silkworm layings from various strains, catering to both traditional and non-traditional silk-producing regions, the Central Silk Board initiated the National Silkworm Seed Project (NSSP). More recently, the Central Silk Board established the Silkworm Seed Technology Laboratory (SSTL) in Kodathi, Bangalore. This facility is dedicated to researching technologies for the production, preservation, storage, and distribution of superior-quality silkworm seeds across all silkworm strains.

Silkworm layings are called seeds and they are of two types

1. Reproductive seeds: Reproductive seeds are those that are used for the purpose of producing the parents of the seeds used for commercial seeds. Since the purpose of the production of this kind of seed is for maintaining the racial purity, they are produced only in special breeding centres by technically qualified personnel. These seeds are often multiplied in number in a series of breeding centres called Breeding Stations.

2.Industrial seeds: The industrial or commercial seeds, on the other hand, are produced in mass to be supplied to the rearers. The race of the parents, the date of egg laying, the

expected date of hatching and the certificate that they are disease-free should accompany these seeds. The commercial seeds are produced in special organisations called grainages.

Breeding stations

These stations serve as the primary sites for the production of reproductive seeds. Reproductive seeds typically consist of pure lines derived from both local strains and high-yielding races, occasionally incorporating F1 hybrids for the generation of double-cross commercial seeds. Within these breeding stations, the prospective parents intended for commercial seed production are nurtured. However, the sheer quantity of purebred parents required for grainage exceeds what can be feasibly obtained through a single round of multiplication from the germplasm stock. As a result, a systematic approach involving three stages for bivoltines and four stages for multivoltines is implemented. Each stage of multiplication is conducted in distinct stations to maintain racial integrity and prevent cross-contamination between strains.

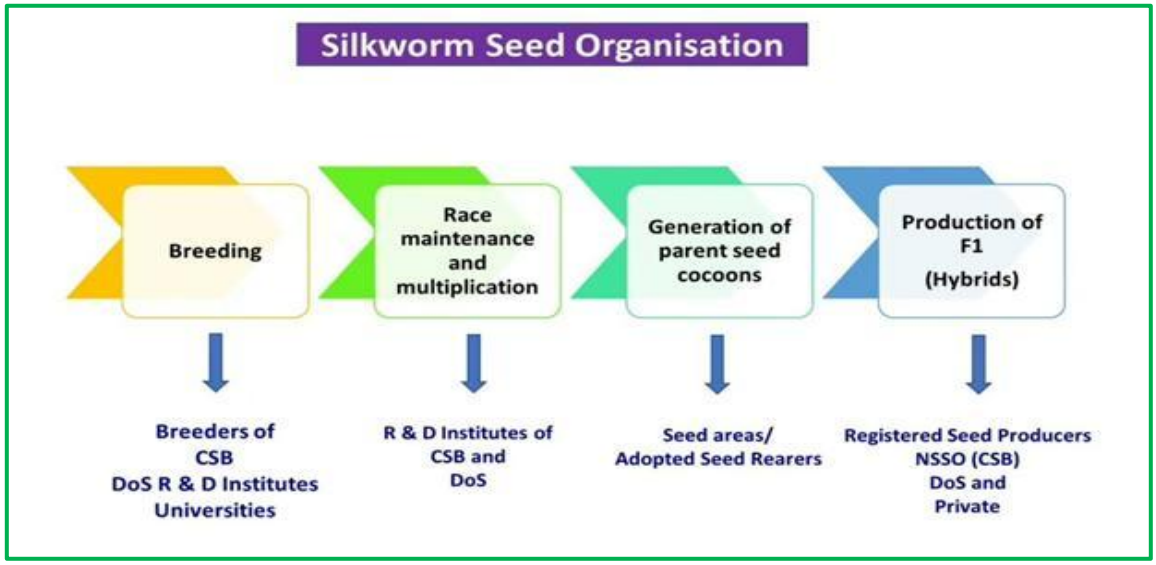

Fig. 1 Silkworm Seed Organisation Flow chart

P4 AND P3 STATIONS (Great Grand Parent Station): Normally, the seeds of multivoltine mulberry silkworm races (whether cross-breed or pure breed) are multiplied in a three-tier system starting from the P3 stations. But exotic pure breed races of bivoltines, as well as, rare multivoltine races have a four-tier multiplication systems beginning with P4 station. These stations are also called as the Race Breeding Stations. They are also the major germplasm banks of the races. They are under Government control where the parental races of the commercial grainage seeds are multiplied. They rear only pure breeds and no hybridisation is done except for research purposes.

Different pure breeds differ in their resistance to diseases, tolerance to environmental conditions, response to the variety of mulberry and other factors. Rearing of each race is done separately by what is called the cellular method of rearing. Each laying of each race is reared separately in different trays, each is fed with the mulberry variety suitable for it and kept at temperature and humidity conditions ideal for it and mounted and harvested separately. There is no mass rearing of even the same race.

As this requires knowledge of the physiology of each race, rearing is done only by technically qualified persons. 25% of the best cocoons are selected and sent to the station at the next lower level for multiplication there. The remaining cocoons are used in the station itself for preparing eggs called **Breeder's Stock Egg.** The P4 and P3 stations are located at the cooler parts of the country.

P2 Stations (Grand Parent Station): These stations not only produce Foundation Stock seeds but also function as germplasm banks. They receive seed cocoons for rearing from the breeding station above them, namely the P3 station. At these stations, the emphasis lies solely on the multiplication of pure breed races, with no hybridization occurring. Each laying from the mother moth is meticulously reared individually and kept separate through the cellular rearing method, ensuring the preservation of racial characteristics without any risk of mingling. Furthermore, the P2 stations meticulously select 25% of the best cocoons from each laying and forward them to the subsequent lower station for further multiplication. The remaining cocoons are either utilized to replenish the existing stock or for silk reeling purposes.

P1 STATIONS: These are the stations where the reproductive seed cocoons required by the grainages are reared. They receive seeds from the P2 stations. The seed cocoons are mass reared and the first hybridisation of double crosses is done here and the hybrids produced are called Foundation hybrids. They send 25% of the cocoons produced to the grainages or to seed cocoon rearers for rearing the seed cocoons which act as parents for the commercial layings.

Selection criteria for Seed Cocoon Rearer

Since the seed cocoons must be healthy, hygienic and preserve the racial characters, its rearing requires technical skill. Seed cocoons are generally raised in P1 stations. Trusted highly skilled farmers who are given special license for this and who get a premium price (50% higher than the commercial cocoon) for their seed cocoons. The choice of the rearer is made after taking into consideration the following:

1. The rearer should have a scientific knowledge of grainage operation and silkworm rearing (preferably undergone a special training) and have an interest in sericulture and should co-operate with the grainage personnel.
2. The mulberry gardens should be cultivated following the new package of practices and should supply good quality leaves.
3. The rearing house should be located in an area suitable for pure breed rearing, with facilities for providing optimum rearing conditions. It should be free from germs of silkworm diseases and have an easy access to the grainage which it supplies.

GRAINAGES

Places where the disease-free layings of silkworm called industrial seeds are produced on a mass scale and supplied to commercial rearers.

The location of a grainage must be:

1. It must be in a cooler area of the locality.
2. It must be situated in the centre of the sericulturally active area.
3. It must have the following facilities:

- Rearing house and mulberry gardens to rear parental seed cocoons
- Facility for procuring and storing seed cocoons from seed cocoon rearers.
- Sex separating and pupa storage rooms
- Moth emergence and coupling rooms
- Egg laying room
- Incubation room
- Cold egg storage room
- Moth examination room.

4. It must have trained rearers to rear the seed cocoon races or 4 have trained extension workers to supervise and assist the seed cocoon rearer.

Procedures in Grainage

1. Rearing of Parental Seed Cocoons: Procuring and storage of seed cocoons from the seed cocoon rearers should be done without subjecting the cocoons to drastic fluctuations in temperature and humidity and without causing any damage to cocoons during transportation. The rearing of parent races to be used for seed preparation should be synchronised by adjusting the time of brushing larval and pupal period in order to synchronise the date of moth emergence of both the parents. The concentration should be on the robustness and health of the parent so that its fecundity is increased rather than on its commercial cocoon characters.

2. Seed Cocoon Preservation: Seed cocoons have to be properly preserved before moth emergence. Care should be taken regarding the following points:

- During transport more shaking should be avoided as this reduces the percentage of eclosion and fertility of the moths
- Cocoons should be preserved in well-aerated and ventilated room. Strong winds and direct sunlight are to be avoided.
- The storage room should maintain a constant temperature of 23-25° C and a RH of 75-80% in order to ensure uniform growth and simultaneous eclosion.

3. Separation of Sexes: In order to avoid self-breeding within the races, and for easy picking of the male and female parents, the two sexes of each race are separated prior to moth emergence. The methods employed for sexing or separation of sexes are:

Sex Separation in the Larva: In rearing races with sex-linked traits, larvae are segregated in the fifth instar, reared individually, and then harvested and transported. Japan uses specific cuticular markings for female separation. Some Indian hybrid races can also be sexed in the larval stage, but this method isn't suitable for many local races.

Sex Separation in the Pupa: To separate female and male pupae, cocoons are manually cut open and examined, then stored separately. With manual cutting, one person can isolate 12,000 to 15,000 cocoons in eight hours, while automatic machines can separate ten times more in a day. In Russia, a sex separating machine is used based on the weight difference between female and male pupae. Pupae can be stored in a refrigerator (3 days for female, 7 days for male) to synchronize moth emergence. Pupal colour changes indicate moth emergence and the compound eye turns black halfway through the pupal period, and the pupal body turns black the day before eclosion.

Fig.2 Cutting of silkworm cocoon for pupa separation

4. Moth emergence: Moths emerge in response to light in the early hours of the morning 12-14 days after spinning. The cocoon/pupae preservation room should be kept in dark one day prior to emergence. On the day of emergence, bright lights are switched on early in the morning (*i.e.*, by 6 a.m.). Moths emerge from 6 a.m. to 8 a.m. Moths of a lot emerge in about 3-4 days. At about 9 a.m. the emerged moths are collected and the room once again darkened. Healthy moths can be collected from the paper cover easily. The female and male moths are sprayed with different harmless colours for easy operation.

Fig. 3 Silk Moth Emergence

5. Pairing and Oviposition: Female and male moths of the specific cross should be

gathered and allowed to pair. The males are broadcast on a tray of females. The moths have tendency to mate immediately after emergence. Mating should not be of less than 2 hours or more than 6 hours duration. After six hours the males are separated by holding the female and twisting the male gently without injuring the external genitalia of the female. Male moths can be cold stored at 7-10°C for use for a second mating for 2 days or stored without mating for 7 days at the same temperature till the females emerge.

Fig. 4 Pairing and oviposition of moths

6. Methods of Industrial Egg Production

a) Cellular bag method: In this method, a coupling pair of moths is introduced into a small bag of perforated parchment paper or paraffin paper or cloth bag and after the eggs are laid, the female moths are examined for pebrine infection. The bag containing the eggs of diseased moths are destroyed.

b) Cellular card method: In Indian grainages, the Japanese method is employed, utilizing craft or card paper divided into 20 squares in 4 rows. Each square accommodates a coupling pair of moths under a funnel plate. After egg-laying, females are removed and examined for disease. Infected eggs are removed and destroyed, ensuring mass-scale egg production.

c) Flat card method: About 40 pairs of coupling moths are allowed to lay eggs on the rough side of a flat card. After egg laying, sampling method is used for mother moth examination. If a certain specified percentage of the sample is found to have pebrine, the entire lot is discarded.

7. Mother Moth Examination: The purpose of mother moth examination is to ensure that eggs supplied are free from trans-ovarial infection of pebrine spores. In the case of uni/bivoltine races, the diapausing eggs are stored and the moths are preserved and examined at leisure. But multivoltines lay non-diapausing eggs which are incubated immediately and moths have to be examined immediately before the eggs hatch out.

Mother moth examination is classified into three types:

1) Individual moth examination
2) Sample moth examination and
3) Mass moth examination.

The choice of the method depends upon the quantity of seeds produced in that grainage and on the availability of technical personnel and equipments.

Diapausing and Non-diapausing Eggs

Eggs of silkworm are of two types:

1) Diapausing eggs: Univoltine races lay only diapausing eggs.
2) Non- diapausing eggs. Multivoltine races lay only non-diapausing eggs.

DIAPAUSING EGGS

Diapausing eggs are either stored till the next season (if mulberry is not available) or awakened from the diapause artificially (if mulberry is available for rearing).

Storage of Spring Eggs

1. Between June and August for 60 days the eggs are stored at an aestivation temperature of 23-25°C for inducing stable diapause.
2. The eggs are allowed to remain at a room temperature (about 20°C) till September or early October and then gradually lowered to 5°C in December.
3. The eggs are washed and kept at 5°C for 50-60 days. This treatment awakens the embryo but arrests development.

4. After this, in Jan/Feb, the eggs are transferred to a still lower temperature of 2.5°C for 40-60 days. Eggs may be stored in this temperature for any length of time.
5. The special intermediate care temperature is to be given in March i.e., eggs are to be kept at a temperature of 10-15°C for 4-5 days.
6. Eggs are to be stored at 2.5°C till taken to incubation.

Storage of Autumn Eggs

These are stored only till next spring. Duration of storage is shorter.
1. Eggs laid in August are kept at 23-25°C for 20 days.
2. The temperature is lowered gradually (*i.e.*, 2.5°C per 3 days) till natural temperature is reached.
3. The temperature is lowered as for spring eggs and stored till needed.
4. No intermediate treatment is needed.

ARTIFICIAL BREAKING OF DIAPAUSE

1. Common Acid Treatment (Hot HCl Method)

This is the most common method used for breaking diapause in commercial hybrid eggs. The treatment is carried out about 20-24 hours after oviposition at 25°C and hence it is also called hot acid method.

Procedure:

1. The eggs are dipped in dilute HCl (specific gravity of 1.075 or 15% concentration) heated to 46°C for about 5 minutes. This treatment stops the eggs from entering into diapause. The HCl has to be washed away with water.
2. Eggs laid on cards must be firmly fixed to each card. This is done by washing in 2% formalin for 5 minutes and washing in tap water and drying in shade.

2. Cold Acid Treatment

Eggs preserved at 25°C after 15-20 hours of oviposition are dipped in HCl of 1.110 specific gravity at 15°C (about 20% concentration) for about 50 minutes. Dipping time varies according to the race, the age of the egg and the temperature of the acid. They are then washed in water.

3. Acid Treatment After Chilling

Eggs oviposited at 25°C are stored at the same temperature for 40- 50 hours. They are transferred to an intermediate temperature of 15°C for 6 hours. Then, they are stored at 5°C and 75-80% R.H. for forty days and then stored at 2.5°C. Before acid treatment eggs are to be brought to room temperature at least 3-6 hours earlier. Then they are dipped in HCl of 1.100 specific gravity at 15°C, and heated to 48°C for 5 minutes. Acid-treated eggs may also be stored in cold room.

Incubation of Eggs

Incubation aims to ensure uniform hatching on a specified day by maintaining eggs at an optimal temperature of 25°C and humidity of 75-80%. Before incubation, stored hibernating eggs require disinfection with 2% formalin. Acid-treated eggs, however, can be directly incubated without prior disinfection. Additionally, the incubation room must undergo thorough disinfection with 2% formalin before use.

There are three methods of incubation:
1. Constant Temperature incubation
2. Raised Temperature incubation
3. Embryonic Method of incubation.

Transport of Eggs

During this transport, eggs should not be exposed directly to sunlight, wind and rain. Eggs may be transported in

- Aestivation period

Fig.5 Transportation Bag of Silkworm eggs

- Hibernation period
- Post-hibernation or Pre-incubation period.

Transportation of the diapausing egg is recommended in the aestivation period. Eggs may be transported for short distances in the hibernation period and transportation should be avoided in the pre-incubation period.

Conclusion

Sericulture industry's success hinges upon the meticulous processes involved in grainage management, seed cocoon production, and egg incubation. These practices ensure the maintenance of racial purity, disease prevention, and optimal conditions for silkworm development. Through initiatives like the National Silkworm Seed Project and the establishment of Silkworm Seed Technology Laboratories, efforts are underway to enhance the quality and efficiency of silkworm seed production. Furthermore, the adoption of advanced techniques such as sex separation and egg treatment methods contribute to the sustainable growth of the industry. Overall, by prioritizing quality, hygiene, and innovation, the sericulture sector continues to thrive and meet the demands of the global market.

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