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A MULTIPRONGED APPROACH FOR IMPROVING SEED AVAILABILITY DURING COMMERCIAL REARING SEASONS IN MUGA ECOSYSTEM

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ABSTRACT

Availability of quality seeds in muga culture for commercial rearing during May-June and Oct-Nov seasons is the biggest challenge, which severely affects the total muga silk production. Both the commercial seasons are preceded by seed and pre-seed seasons with highly unfavourable weather conditions, which affects the availability of quality Disease Free Layings (DFLs) due to low cocoon yield, reduced fecundity, poor hatching and male sterility. Therefore, there is an urgent need to develop an integrated approach to improve the availability of quality DFLs during commercial seasons. Towards this end different strategies are being developed and tested. Breeding for selecting muga lines with high fecundity has been taken up. To avoid unfavourable weather conditions during seed crops especially during summer months June-September, efforts are being made to identify the cooler regions in North-eastern states and to take up seed rearing in the selected regions. Wild muga that exhibit winter hibernation are being studied to identify the linked gene and to introgress it into cultivated population to completely avoid rearing during winter season. A dual-purpose cold reeling procedure has been developed, which has also been tested field level, to reel the silk and obtain live pupae for use in grainage. Through biotechnological approaches the realized fecundity has been increased. Improved mountages that provide enough space, aeration and reduced temperature are being tested for increasing the number of good cocoons and also reducing the male sterility due to high temperature. Other approaches such as rearing of seed cocoons in non-traditional areas having muga host plants such as Uttarakhand state, cold preservation of both DFLs and cocoons, new plantations in cooler regions, effective utilization of grainages developed under different government schemes through state governments, etc., are being explored to effectively increase the quality DFL production for use during commercial seasons in muga ecosystem. These approaches are of great promise and help in achieving 'Sustainability Development Goals 2020' of Central Silk Board.

Keywords : DFLs, vanya silkworms, muga, breeding, genetics.

Introduction

An important factor contributing to the success of sericulture industry is not only augmentation of silkworm food plants but also ensuring timely supply of superior quality of silkworm seed to the farmers. Healthy egg production is indeed the primary requisite for conducive growth of the entire silk industry. Timely supply of quality silkworm seed can alone sustain sericulture as a commercial crop in competition with other cash crops. The quality silkworm seeds are from the layings that are entirely free from diseases and also have higher percentage of viable eggs, high hatching percentage and assure a stable crop. The availability of quality DFLs has been a major challenge in case of muga culture, where supply of DFLs during commercial crops is a chronic issue.

The muga silkworm, *Antheraea assamensis* is a highly, heterogeneous unique and semi-domesticated species of Saturniidae family of the insect order Lepidoptera, endemic to Assam, adjacent foot hills of Meghalaya, Nagaland, Arunachal Pradesh, and Mizoram states of North-east India; but even during favourable season 30% of the farmers fail to

make full utilization of the available plantation due to shortage of seed cocoon and only quality muga silkworm seed in sufficient quantity will make possible the way for restoration of the past glory of muga culture of Assam (Samson and Barah, 1989). Due to low yield of seed cocoons it has become difficult to produce the required quantity of cocoons for production of targeted DFLs for supplying to the commercial rearers (Sahu *et al.*, 1998).

Muga silkworm is multivoltine and there are six crops in a year with two commercial crops. To raise either of the two commercial crops, four seed crops have to be raised in two separate rearing cycles (Khanikor and Dutta, 2006). Multivoltinism is one of the major problems for which the maintenance of different seed broods of muga silkworm is difficult, time consuming, laborious and hazardous making the unavailability of good quality seed cocoons in specific seasons. One of the major factors affecting the muga silk industry is the gap in seed cocoon production as the seed crops always fall either in the hot and humid summer or in extreme cold and foggy winter making these crops uncertain (Saikia *et al.*, 2016).

The muga raw silk production has not shown significant growth and has stagnated in the past three years (Figure 1). The fundamental reason behind the poor production is shortage of quality muga seeds for commercial rearing. Non-availability of quality seed in required quantity and in the proper season, for conducting the commercial crop rearing has been attributed to be the major factor in the stagnation of muga silk production. The issues in seed availability in muga are because of many reasons specific to muga sericulture, which are listed below.

- 1) Unfavourable climatic conditions during seed crop especially during summer (sterility, poor hatchability and reduced fecundity).
- 2) Inherently lower fecundity in muga silkworm in general (140 eggs per DFL)
- 3) Non-adherence to standard protocol during cocoon storage, grainage and egg incubation.
- 4) High demand for muga silk filament leading to farmers stifling the cocoons and selling them for silk extraction.
- 5) Absence of egg diapause and pupal hibernation in cultivated varieties of muga silkworm.
- 6) Lack of training in rearing and grainage for farmers/graineurs.
- 7) Preparation of seeds by farmers themselves without testing for pebrine.

Taking cognisance of these issues at hand, this article discusses different strategies that can be applied to mitigate seed availability problems in muga ecosystem. A comprehensive and concerted approach involving different techniques and interventions would help address this problem. If these strategies are implemented properly, the seed issues in muga ecosystem may be mitigated and the total muga silk production may be increased to meet the set target for the year 2030.

Strategies to mitigate seed availability issues in muga ecosystem

Finding effective solution to complex problems is not easy, but by using the right process and techniques, one can be more efficient in the process and address the problems easily. Solving challenges is easier with the right methods. Create innovative solutions and solve tough problems fast with these problem-solving methods. The seed availability problem in muga culture requires a multipronged approach that takes into consideration all the hurdles that affect seed availability. Some of the approaches that can be taken up are explained here.

1) Rearing of muga silkworm in cooler regions during summer months

One of the major challenges for muga rearing is supplying good quality seeds in Oct-Nov commercial crop season. As this season is preceded by harsh summer where most of the areas in Assam state are very warm and humid, there is a high chance of complete crop failure due to heat induced mortality and higher disease incidence due to increased susceptibility to diseases. Even if cocoons are formed during summer season, they cannot be used for DFL production as the emerged moths may be sterile and would produce eggs that do not hatch.

In order to address this issue, the CSB has taken up remedial measures through identification of cooler regions within Northeast India and developing some plantations. These plantations have been developed under different schemes and are now ready for rearing. Several areas in Nagaland that are situated in higher altitude like Mokokchung, Wokha, etc., can be utilized for summer rearing (Figure 2). The seeds produced in these areas are sufficient for commercial rearing in Oct-Nov season in the state of Nagaland. Many areas in the state of Mizoram are cooler during summer. Hot and humid climatic condition of Mamit sub-division located at an altitude of 2978 m above sea level in Mizoram has been reported to be quite sustainable for muga culture (Yadav and Samson, 1987). The availability of naturally grown Soalu trees makes it more remunerative to utilize these regions for summer rearing. Towards this end the CSB has identified plantations for taking up seed rearing. Also, the lack of training to farmers in the state of Mizoram has been realized and training programmes are being conducted to impart training in muga rearing and grainage activities. Further, Northern region of West Bengal such as Kalimpong and some areas in the state of Sikkim, where the temperature will be low during summer have also been earmarked for seed rearing. A test rearing in these areas has provided promising results.

Systematic planning of rearing during summer season in cooler areas and strict adherence to rearing technology will be most useful for improving seed availability during Oct-Nov commercial season.

2) Standardizing the grainage protocol for maximizing the overall fecundity

An analysis of grainage performance showed that influences of environmental factors have strong reflection on fecundity and hatchability. Grainage performance during the pre-seed and seed corps suffer due to low humidity and low temperature (Dec-Jan) and high temperature ranging from 26-36°C and high humidity (74-76%) during summer season resulting in requirement of more number of cocoons for producing one DFL (cocoon : DFL ratio 5: 1 or even more), poor moth quality and low to very low hatching (0-25%) due to exposure of the late age worms to high temperature (36°C) during out door rearing and spinning under indoor condition. This has been identified as one of the major problems in muga culture that causes shortage in production of main commercial seed during kotia crop (Sahu, 2004). Strict adherence to standard operating procedure in grainages, maintenance of optimum temperature and humidity, especially disinfection operation leads to higher seed yield. Stringent measures in mother moth examination and exclusion of diseased eggs, will ensure quality DFLs, which in turn help in better crop and higher cocoon yield. Training of graineurs in scientific grainage procedure will also be helpful in reducing common mistakes in grainage.

Cold preservation of muga cocoons has been tried and it has been found that incubation or storage of seed cocoons at low temperatures can help avoid harsh summer season up to certain extent. A study to develop a suitable long term seed cocoon preservation method for skipping unfavourable pre-seed crop by postponing moth emergence. Eight days old muga silkworm seed cocoons were subjected to the low temperatures at 7.5±1°C and 10±1°C for long term preservation and the results indicated that muga seed cocoons

can effectively be preserved at $10\pm 1^{\circ}\text{C}$ for 42 days without affecting the grainage parameters measured for seed production (Rajkhowa *et al.*, 2011).

As the muga seeds do not undergo diapause, low temperature preservation of seeds to arrest the developmental process for some time to skip the adverse environmental condition may overcome the constraint. An egg incubation schedule has been formulated for obtaining uniform hatching, where 4 days eggs are preserved at 12.5°C for 9 days to prolong the embryonic period by 22 days (Rajan and Hajarika, 2012). Effective implementation of cold preservation technologies during summer season by employing cold storage facilities developed by government agencies including those developed mulberry silkworm seed preservation would help save the seed cocoons without losing the viability.

3) Breeding to increase potential fecundity

Muga silkworm has the potential to lay a good number of eggs (250-280) but realized fecundity (120-150) is comparatively poor even during the favourable seasons compared to eri (440-470) and mulberry (450-550) (Dutta *et al.*, 2013). Owing to continuous exploitation without proper breeding plan and inadequate seed selection norms, there is apparent deterioration of quality as well as quantity of muga seeds. It is well known that the success of sericulture depends on production of sound seed with good vigor and therefore there is a need to improve the productivity through enhancing fecundity in the cultivated muga race. The egg laying potential or the realized fecundity could be improved through adoption of appropriate breeding plan. In view of this, efforts are underway in CMERTI to isolate muga lines with high fecundity. The average fecundity is around 140 in cultivated lines. However, in case of wild muga the fecundity is recorded to be more than 300 (Unpublished data). Through mass selection, lines are being selected that have higher fecundity. Even if the fecundity is increased by a margin of 10%, the total production can be increased by 25MT as sufficient host plants available to accommodate increased seed availability.

4) Breeding for incorporating pupal hibernation in cultivated varieties

Diapause / hibernation is an arrested state of development which is pre-programmed, that allows animals to save themselves from the harsh environmental conditions. The expression of hibernation behaviour sets in during unfavourable environmental conditions, such as winter, extreme summer, periods of drought and season in which appropriate food is not available (Tauber 1986). Diapausing insects become highly tolerant to cold, heat, desiccation and starvation (Masaki, 2002). Diapause occurs in genetically determined stage(s) of metamorphosis which are species specific and is represented by low metabolic activity. Photoperiod plays a major role in diapauses induction in insects, and temperature is typically seen as one of the possible modifiers of the photoperiod responses (Mc Nell and Fields, 1985). Low temperature is reported to be the reason for diapause induction in tropical insects, high temperatures blocks initiation of development and the individual remain in quiescence stage and the diapause can be terminated only when temperature is lowered (Denlinger, 1986). This mechanism is used to maintain diapause during the hot dry season and development is triggered with cool rainy season.

The onset of rains is frequently linked to diapause termination and may account for the rapid increase of insects (Bowden, 1976). In tropical insects, termination of diapause is temperature dependent. Lower temperature results in longer diapause.

In muga probably due to continuous selection for non-hibernating trait, pupal hibernation is not observed in cultivated stock. Therefore, the farmers and government agencies have to continuously rear muga silkworms throughout the year even in unfavourable weather conditions. This has led to a lot of problems including the seed availability. However, pupal hibernation seems to be present in wild muga. The wild muga collections exhibit both winter as well as summer hibernation (Unpublished data). The winter hibernation is longer and starts in the month of November and moth emergence starts in April month (Figure 3). The summer hibernation sets in June and the moth emergence starts in October. Harnessing this behaviour for commercial rearing will significantly help in improving the seed availability in muga ecosystem as hibernation will aid in skipping rearing during unfavourable months.

However, the genetic loci linked to this hibernation behaviour is not yet identified and efforts are being made using large scale genotyping and comparative genomics analysis to identify the gene(s) linked to hibernation in muga silkworm. Identification and introgression of hibernation linked genes into cultivated stock will go a long way in addressing the seed availability problems in muga ecosystem.

5) Host plant volatiles in improving the realized fecundity in muga

The aim to find favourable and secure conditions to benefit the offspring is ubiquitously spread among the living beings. The parents seek to provide protection from a manifold of risks like predation or starvation. While many species take care of their offspring after hatching, parents of most insect species are dead before their offspring hatch out of eggs. Thus the choice of adequate oviposition sites and hosts for the comparatively immobile offspring becomes vitally important for female insects to increase offspring survival, reproductive success and consequently, maximize inclusive individual fitness. To identify suitable host plant for oviposition, the female insects depend on olfactory or visual cues (non contact) or gustatory or tactile cues (with contact). Among the chemical senses, olfaction plays a key role in host location for many insects. The discriminating oviposition behaviour depending on host species and condition is facilitated by a highly sophisticated olfactory system, which reliably recognizes volatile signals (Spathe, 2013). This behaviour of insects has been harnessed in sericulture to enhance realized fecundity in muga silkworm by using sprays of host plant volatiles extracted from different host plants of muga silkworm.

Under the project on development of technology for enhancing egg laying in vanya silkmths by application of host plant volatiles, several volatiles have been tested and it has been found to enhance the realized fecundity (Annual Report, CMERTI, 2020-21). Impact of leaf extracts of different host plants on realized fecundity and egg retention in Muga silk moth has been studied in this project. The results showed that muga moths treated with Soalu leaf yielded maximum fecundity. Negative impact was found

when mother moth was treated with Mejangkari leaf and recorded lowest fecundity.

Realized fecundity is less during winter season in muga moths, which leads to reduced seed production in grainages. Application of specific host plant volatiles would help increase the fecundity and thereby total seed production improves and it in turn eases seed availability during April-May season. Even if there is an increase of 10% in realized fecundity, it would lead to increase in production of at least 25MT muga silk.

6) Use of cold reeling technique in commercial reeling

The muga cocoons are bought by the reelers for a premium during seed and pre-seed crops. This will decrease the availability of muga cocoons for seed production, which eventually affects the seed availability during commercial season. This is a vicious cycle that happens every year and farmers' demand for seeds during commercial season could not be met and this affects the total production even though there is a plenty of som and soalu plantations available for rearing during commercial season.

To address this issue, a new cold reeling procedure has been developed at CMER & TI, Jorhat (Central Silk Board) (Unpublished data), wherein the cocoons can be reeled at room temperature without the requirement of stifling. This procedure retains the live pupae while reeling thereby yielding both reeled silk yarn as well as live pupae for DFL production (Figure 4). The technology has been tested both at lab level and also at commercial level. The reeled silk is as good as hot reeled silk yarn. There is no significant effect on grainage and DFL production from cold reeled pupae.

The technology has several advantages as listed below:

- a) Reeled silk as well as DFL can be obtained from cocoons, providing additional income for the farmers/reelers/graineurs.
- b) In unfavourable seasons the cocoons are bought for premium and silk is reeled leaving very few cocoons for DFL production. Cold reeling will help in improving the availability of DFLs during unfavourable seasons and seed availability improves in commercial season.
- c) In normal DFL production, how many live pupae are there inside the cocoons cannot be deciphered easily. Through cold reeling, the exact number of live pupae can be enumerated. Therefore, the total number of DFLs that can be procured from given lot of cocoons can be estimated.
- d) Number of males and females can be enumerated from cold reeling. This will help in proper planning of grainage. If one sex is less, then the pupa can be exchanged with others who have more of that sex.
- e) It leads to increase in total silk production without increasing the DFL rearing, as the cocoons which otherwise would have been rendered as cut cocoons will be reeled and silk yarn is produced.
- f) This will improve seed availability in other North-eastern states neighbouring Assam, which currently depend mainly on Assam for seed supply. The states like Meghalaya, Nagaland, Mizoram, Arunachal Pradesh and Manipur can produce the required DFLs in their state itself and also reel the muga silk.

- g) As green cocoons are used for cold reeling, the stifling and cooking is avoided, which saves time and money to the reelers.

7) Experimental rearing in non-traditional areas (North and Southern states)

In order to prepare for ill effects of climate change and to make the muga seeds available for commercial season, it is better to explore new areas outside of Northeast India where the months of June-September are relatively cooler and months of December-February are relatively warmer. Towards this end a few efforts have been made to identify new areas. There is enough scope for extension of muga culture in the hills of Dehradun because due to adverse climatic condition the rearing of muga silkworm particularly the seed crops are very uncertain in northeast. In newly explored areas, during the seed and pre-seed crops the scenario is not as poor as Assam. Another area that can be explored is in West Bengal particularly Cooch Behar district where the climatic contiguity with Assam explores successful muga culture. Upon preliminary observation (Ray, 2003), it can be said that October-November is the main commercial crop-rearing season. Surprisingly during March-April the seed crop-rearing season, the fecundity observed 162.50 much higher than Assam. Such areas can be exploited for production of seed crop during March-April months.

Several som plantations have been developed in Kalimpong district of West Bengal district and also in Sikkim state. These plantations can be effectively utilized for producing seed cocoons during August-September months when the rains subside and the temperature is relatively low. The farmers in this area need to be trained well in rearing and grainage techniques (Figure 2). Further, the Sericulture department of the state of Uttarakhand has surveyed and identified a large population of muga host plants, including *Litsea monopetala* and *Persea bombycina* in Bageshwar and Nainital. These areas can be effectively utilized for summer rearing to produce seed cocoons for commercial season of Oct-Nov. Also, as winter is less severe in southern part of India, som plantations may be developed on trial basis in a few areas and experimental rearing can be taken up in Dec-Feb months to see if good cocoon yield can be obtained. Expansion of muga culture beyond Northeast India mainly for the purpose of production of seed cocoons would greatly help addressing the seed availability issues during commercial seasons.

8) Proper mounting of mature worms for spinning

The spinning of cocoons is a crucial phase in vanya silkworm rearing. The time and method of mounting as well as the cocooning frame, otherwise called as 'mountage', are the most important factors influencing the quality of cocoons and thereby, the raw silk yield and quality. Even if the silkworm crop is healthy, improper mounting methods, spinning conditions, mounting density, mounting of pre or over matured larvae and poor type of mountages can result in inferior quality cocoons. Such cocoons yield low quality seeds affecting the next crop. In muga, traditional jail type mountages are being used by farmers for mounting matured worms. Aeration is a problem in jail mountage during summer months and when the humidity is high, leading the moist cocoons leaving them prone to fungal infection. The microclimate inside jail mountage during summer is warmer than outside leading to mortality, sterility and infection of pupae. Therefore, there is a need for a better mounting device

that is reusable, less labor intensive, economical, simple to use, increases silk recovery (Figure 5).

To overcome the limitations in Jali based moutage, the plastic collapsible moutage that is extensively used in mulberry sericulture looks to be a viable alternative with a few modifications. Use of plastic collapsible moutage or Bamboo-nylon moutage for cocooning of muga matured larvae require less labor than that of Jali, and is economical as they are reusable for many years. Plastic moutage provides enough space and aeration for matured muga silkworm to weave cocoon. The fully formed cocoon is easy to harvest and is devoid of any dirt. The cocoons harvested from such improved moutages will yield higher seed than jali mounted cocoons.



Fig. 4 : Application of newly developed cold reeling technology provides silk yarn as well as live pupae, which can be used for producing DFLs.

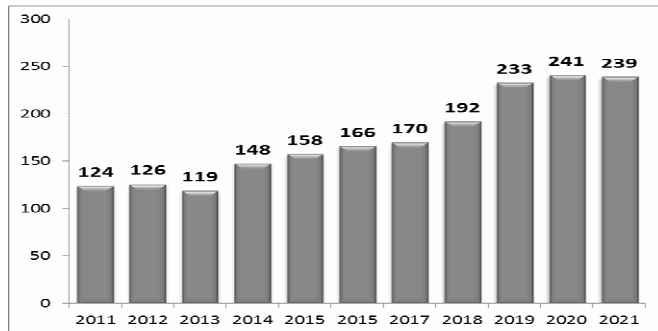


Fig. 1 : Year wise total production (MT) of muga silk from 2011 till 2021. Though there is an increase from the production of 124MT in 2011 to 239MT in 2021, the last three years have seen the stagnation in increase of silk production (Source: CSB, Bangalore).



Fig. 5 : Use of proper mounting devices not only increases silk recovery but also helps in producing quality DFLs. The plastic moutage shown here can accommodate 250 matured larvae and

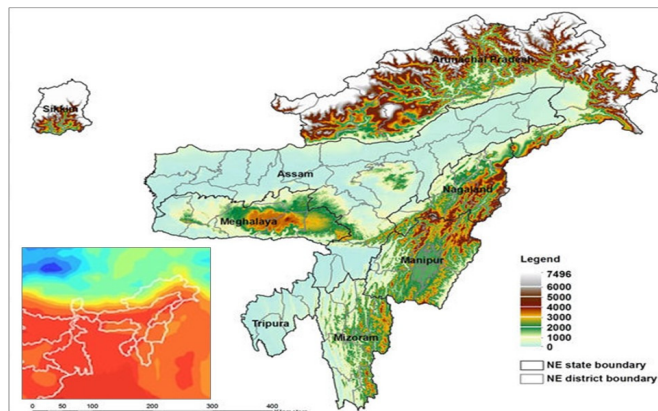


Fig. 2 : Map of Northeast India showing altitude and summer average temperature (Inset). Based on altitude and average summer temperatures, cooler regions can be identified and new som plantations can be developed to produce seed cocoons during summer months of June-September.

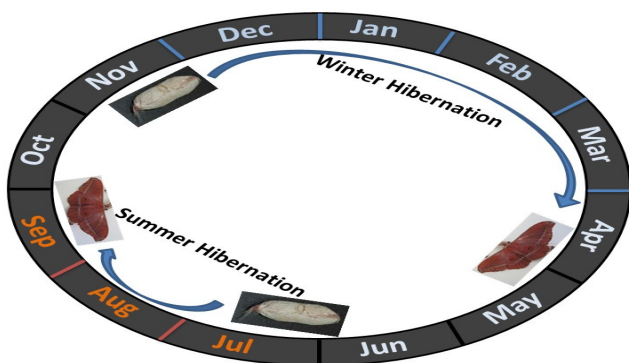


Fig. 3: Breeding for incorporating pupal hibernation in cultivated varieties would avoid rearing of muga in unfavourable seasons.

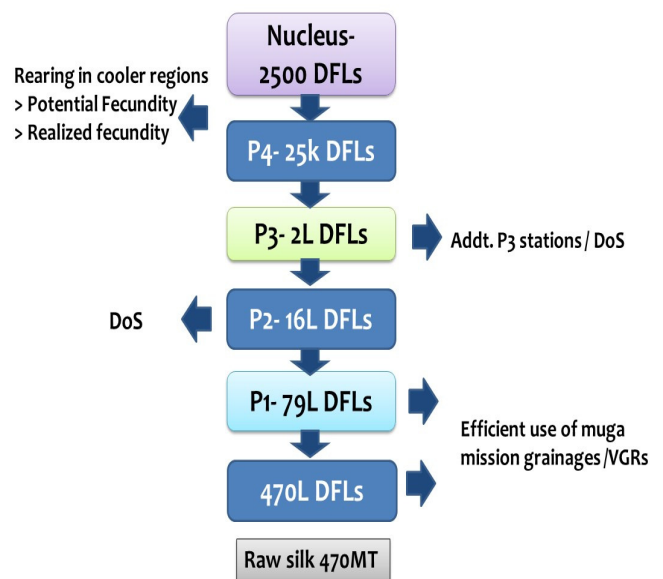


Fig. 6 : Schematic showing different ways to achieve preparation of required muga DFLs for achieving production target of 470MT of silk yarn. A multipronged approach as described will help achieve the target by the year 2030. L; Lakh – 0.1M: DoS; State Department of Sericulture; VGRs; Village Grazing Reserve.

Conclusion

Improving seed availability in muga culture requires a concerted effort, wherein the potential fecundity is improved through continuous breeding efforts, realized fecundity is increased through application of host plant volatiles, more cocoons are made available for seed production through dual purpose cold reeling technology, better quality healthy cocoons are produced through use of better mounting devices like plastic mountages, avoiding rearing during unfavourable seasons through development of summer and winter hibernating muga breeds, following scientific grainage procedure in farmers field through systematic training of graineurs, actively identifying and rearing in cooler regions of Northeast India during summer season, identifying potential regions outside northeast for rearing seed crops, etc (Figure 6). Effective implementation of these technologies and methods holds promise in mitigating seed availability problems in muga ecosystem.

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