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An appraisal of Better Management Practices in Penaeid shrimp hatcheries

Rationale:

The introduction of the alien penaeid shrimp *Litopenaeus vannamei* through Specific Pathogen Free (SPF) broodstocks opened up a new vista in coastal aquaculture of the country. However, shrimp farming activity is plagued by diseases that have their origin in the stress cultured shrimp are subject to the unsustainable practices followed. Production of healthy, disease free seed is one of most important pre-requisite for sustainable shrimp production. *Penaeus monodon*, the major native species cultivated in India, had suffered serious setback because of WSSV outbreaks and one of the main bottleneck is the difficulty in producing disease free seed. In this connection, the various management measures that need to be improved or refined to ensure disease prevention, stress reduction and environment and food safety are discussed in the present publication. Better management practices (BMPs) of seed production and farming systems which are system specific and cost-effective, need to be developed for sustainable shrimp farming. Application of BMP in hatcheries with focus on biosecurity measures, stress free environment and zero tolerance to contaminants and antibiotics would prevent diseases and ensure production of healthy animals which are in accordance to environmental and food safety guidelines. These BMPs would require essential infrastructure, the development of practices which ensure total biosecurity, the provision of adequate amounts of clean water, the responsible use of chemicals, correct feeding practices, and the assurance of the health status of stocks through in-house testing of pathogens. The issues related to hatchery management, the state of management measures followed and the BMPs that need to be in place are discussed below under 15 headings.

1. Issue: Optimal Water quality requirements

Management measures followed:

Breeding and seed production of penaeid shrimps require oceanic quality sea water without pollutants and contaminants. The optimal water quality characteristics are: temperature (^{o}C) 28 – 32; salinity (ppt) - 30 – 34; pH - 8.0 - 8.4; dissolved oxygen (ppm) - above 4; Ammonia - N (ppm) - less than 0.01; Nitrite - N (ppm) - less than 0.01. When these optimal levels are not maintained because of seasonality, monsoon, sub-optimal salinity or pollution, the seed production level will be seriously affected.

Better Management Practice

- Serious considerations for the year round water quality should be given at the time of selecting the site for the hatchery. Areas with optimal water quality for most of the year should be chosen.

2. Issue: Cross contamination between the different production units in a hatchery

Management measures followed:

A penaeid shrimp hatchery consists of different rearing units – quarantine, maturation, larval rearing, and nursery rearing and feed preparation sections algal culture, *Artemia* decapsulation and hatching and wet live feed processing. The operation of the various units is generally interdependent for water and air supply and manned by common personnel and this is one of the major reasons for contamination.

Better Management Practices:

- A properly designed hatchery should consist of physically separate units for quarantine, maturation, spawning, larval rearing and algal (indoor & outdoor units) and *Artemia* and other live feed preparation units.
- The water and air supply systems should be exclusive for each unit.
- Movement of personnel between the units should be avoided and if inevitable, proper disinfection should be done before entry.
- In case of old hatchery without any physical separation, barriers and product flow controls can be implemented to isolate one unit from other effectively.

3. Issue: Supply of pathogen and contaminant free quality sea water

Management measures followed:

Penaeid shrimp hatcheries require clean and clear water and one of the important operating procedures is the water treatment and disinfection protocol. Sea water intake system incorporating various filters and chemical disinfection will ensure that the water entering the hatchery is free from pathogens and contaminants. What is presently practiced lacks a unit exclusive approach for filtration and disinfection involving use of carbon/cartridge filters, UV or Ozone filters, chlorination, EDTA, KMnO4 treatments.

Better Management Practices:

- Each functional unit must receive water that is treated and preferably must have separate water supply systems. If possible, re-circulatory system in specific sections is to be added to ensure water quality with reduced water usage.
- The intake water of suitable quality (29-32 ppt salinity) should be retained in settlement tank for 1-2 days followed by effective treatment with chlorine (10-20ppm for 12-24hrs) followed by sodium-thiosulphate dechlorination. Up to 2 ppm of potassium permanganate (KMnO4) can be added to the settlement tank.
- Activated carbon filter is advisable to ensure no chlorine or dissolved organic residues slip into the hatchery system. Also cartridge filter (1-5 micron), UV or ozone filter are utilized along with EDTA (10-30 ppm) or Treflan (0.05-0.1 ppm) as required for different units like LRT, maturation, *Artemia* and algal units.

4. Issue: Stress and cross-contamination of broodstock during collection and transportation of wild broodstock

Management measures followed:

Normally live broodstock are collected by trawlers or country boat operators. In case of trawlers, the duration of trawling is more than 2 to 3 hours and this leads to high level of stress to broodstock. In some cases the total fishing period will be for more than 2 to 3 days and the shrimps are kept for long duration in on-board in small containers leading to stress and cross contamination between infected and non-infected stock.

The sea water used for transportation of the broodstock in the boat and also at the port collection centre is generally not filtered and is likely to contain pollutants and pathogens. This could lead to contamination of the stock.

Better Management Practices:

- For capture of shrimp broodstock, short duration of trawling should be done.
- The collection and transportation should be stress free and the fishing ground should be as far as from the coast. The captured stock should be immediately brought to the shore. Collection boats could be used to collect from different trawlers and move the broodstock to the hatchery.
- The broodstock after capture should be kept individually in filtered and disinfected sea water.
- To avoid stress to shrimp, they can be kept under oxygen packing in low temperature (18-28^{o}C) and stocking should not be >400g per 8 lit of water. Placing a rubber tube over the rostrum will stop puncturing the bag. To chelate the heavy metal, EDTA 10ppm and to control nitrogenous metabolites, 1g/L activated carbon or probiotics may be added to the water.

5. Issues: Contamination of the hatchery/ maturation system through broodstock

Management measures followed:

Broodstock account for >20% of production cost, largely because of the highly stressed and infected shrimps form considerable portion of the wild catch.

The wild broodstock are generally taken collectively into the quarantine, tested and then taken into the hatchery without using any disinfection. This will essentially lead to contamination of the hatchery system.

Fig:1: Brood Stock Tagging

Better management Practices:

- The hatchery should have a strong wall or peripheral boundaries to reduce any biosecurity risk and contamination of hatchery.
- As far as possible broodstock should be held individually until their disease status for WSSV and MBV is checked.
- For *P. monodon* the female broodstock should be having a weight to length ratio of at least 7.5 g/ cm and preferable weigh at least 220g (>28 cm) whereas the males could be at least 70g (21cm length).
- Upon arrival at the hatchery, the broodstock should be treated with 100 ppm KMnO4 or liquid povidone for 1 min before being stocked in the tanks.
- The diseases check through PCR for WSSV should be done individually for each broodstock by taking a piece of pleopod (cut should be disinfected with liquid povidone iodine) and microscopic observation for the MBV should be done by observing the occlusion bodies in the faeces with malachite green strain.
- The testing for WSSV could be repeated after spawning and if WSSV is confirmed, the entire batch should be discarded after proper disinfection
- If any of the shrimps start dying after checking negative for virus, hemolymph bacterial count should be estimated in the surviving shrimp and if vibrio is detected in significant numbers in hemolymph, probiotics and vitamin C can be given through feed. However shrimp with black melanised lesions in the body or white muscles or are bright red in color should be discarded immediately before these symptoms appear in others.

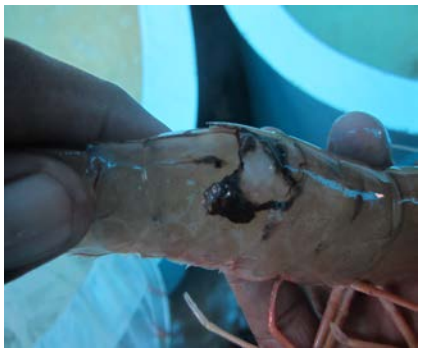

Fig:2: Bacterial disease

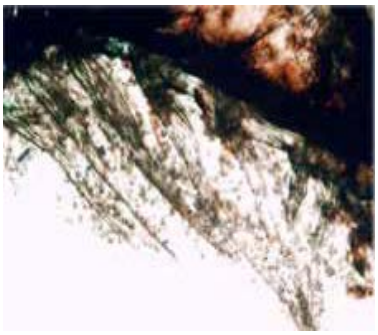

Fig:3: Protozoan disease

Fig:4: Stressed shrimp

6. Issues: Avoiding stress to the broodstock during induced maturation and spawning

Management measures followed:

Stress caused to the broodstock will seriously affect the egg quality and fecundity. The factors that could cause stress during maturation process are the stocking density, water quality and the type of feed given. Often the stocking density of the brooders is not optimum and the quality of feed given are ascertained.

The time and stage of moulting at which the females are ablated decides the latency period of maturation. Hatcheries do not consider the stage and preparedness of animal for undergoing eye stalk ablation (ESA) thus resulting in reduced response and stress induced mortality. Normally water quality and management practices are not properly adhered in spawning and hatching sections.

Better Management Practices:

- Stocking density in the maturation tanks should be restricted to 2-3 nos/sq m for *P. monodon* and 3-4 nos/sq m for *L. vannamei*. Vitamins (vit C -2g/kg and E -0.2g/kg) or astaxanthin (0.1 g/kg) can be mixed with live feed like squid, molluscs to get better reproductive performance and pigments.
- After holding for a week, only inter-moult (hard shell) females kept in chilled seawater (20-25^{o}C), are ablated by cutting one eye with hot pincers or tying with string. Pre-moult or post-moult shrimps can be used after a week or so they can withstand the ablation stress.
- Cartridge and UV filtration systems treated water should be utilized for spawning/hatching tanks
- Immediately after spawning (usually between 8pm-2am), the female should be taken out and placed back into a clean maturation tank. The eggs can be transferred to separate (1/female) 100-200 litre egg hatching tanks filled with clean seawater with 20-30ppm EDTA and 0.05-0.01 ppm treflan.
- Preferably, each female broodstock can be spawned a maximum of 3 times in case of *P. monodon* and 10-12 times in case of *L. vannamei* before discarding in order to maintain high quality nauplii. Unless the female spawn three times before moulting, it should not be used again. Hatching tank should be illuminated with good light source which increases the hatching rate. Only healthy nauplii, attracted to light should be harvested at around noon of the day following spawning, disinfected and stocked in to larval rearing tanks. If a single spawn shows a low hatching rate (<40%), it should be considered unhealthy batch. and discarded

7. Issues: Prevention of vertical transmission

Management measures followed:

Bacteria and viruses which were not detectable in the broodstock may still proliferate and be passed on to the eggs and to nauplii. The egg and other stages are not normally checked for any detectable level of bacterial or viral pathogens. Simple practices like egg washing, disinfection which have been proven to reduce the chances of vertical transmission are not practiced routinely. Hatching is seriously affected by the water quality especially the temperature, salinity and pH. Water quality and aeration distribution in the hatching tank are not given extra attention.

Better Management Practices

- Spawning tanks should be placed in dark rooms without disturbance to the spawners and best quality water should be available for spawning and hatching.
- Spawners should be transferred to the spawning tanks in the late evening and no feeding should be done after that since it will contaminate the eggs at release.
- Mother shrimp should be removed immediately after spawning.
- Eggs should be collected, washed thoroughly and disinfected before disbursing in hatching tanks with fresh filtered and disinfected sea water. The eggs are also being checked for any bacterial and viral infections.
- After hatching is completed, the active nauplii should be collected using their positive phototactic behaviour.
- The collected nauplii should be washed and disinfected with formalin or any other known disinfectant before returning them to the tanks with fresh filtered and disinfected sea water. They should be distributed in larval rearing tanks when they reach N5 stage.

8. Issues: Sub-optimal condition and practices for healthy larval rearing
Management measures followed:

The nauplii is generally stocked at a density between 50 and 100 nauplii per litre (50-100,000/m^3), assuming a full larval rearing tank. However, the uniformity of the stocking is not given importance. This often leads to managerial problem and brings up problems like zoea syndrome in *L. vannamei*.

Better Management Practices:

- Stocking in the entire unit of LRT should not exceed 3-4 days to maximise biosecurity and prevent management related problems.
- Water should be exchanged at 10-30% per day through the 4-6 day mysis period, 30-50% per day from PL1-5 and at >50% per day from PL6 until harvest at PL15. If any disease or water quality problems occur, rate of water exchange should be increased.
- In case of any disease symptoms appears, samples may are to be sent to a PCR laboratory once (2-3 days before harvest) or twice (at nauplius or PL5 also) during the cycle for screening for viral diseases.
- Water exchange and algal feeding follow a pattern as the larvae pass through protozoea, mysis and metamorphose in to post larvae and an optimum schedule should be followed.

9. Issue: Contamination through algae

Management measures followed:
Generally, *Chaetoceros*, *Skeletonema* or *Tetraselmis* spp are used as live feed and every hatchery maintains an indoor and outdoor facility with qualified technician to carry out the work. The outdoor mass culture is the most vulnerable area for the high level proliferation of bacterial population. The quality and quantity of the algae are not given enough importance by many hatchery operators till there is a problem in larval rearing. Pure strain which can ensure the quality are not maintained by the hatcheries and most of the time the mass culture is not started from pure culture by scaling up the volume gradually. Outdoor algal tank often gets contaminated and algal crash and alternative strategy during failure in outdoor algal unit especially during cloudy season are not readily used.

Better Management Practices:

- For zoea, the best feed is clean live algae (*Chaetoceros* sp, *Thalassiosira* sp or *Skeletonema* sp.), fed at 80-130,000 cells/ml. Alternatively, dried Spirulina and liquid or powdered/ microencapsulated dry diets (10-80 microns in size) can be fed every 2-4 h if live algae are not available, especially during failure in the outdoor algal unit.
- The algae in full bloom are concentrated through filtration through a fine mesh bag and the concentrate added to the larval rearing tanks. The final cell densities (for *Chaetoceros* sp.) should be at 80-130,000 cell/ml for zoea and mysis (peaking at Z3), declining to 50-60,000 cells/ml during early Pl stages (optional)
- The pure culture must be screened from time to time to avoid any contaminations which otherwise creeps in unnoticed and latter becomes a problem.
- Outdoor tank contamination should be avoided by maintaining good water quality, preventing air borne dust and through use of proper nutrients and inoculum.

10.Issue: Contamination through *Artemia*

Management measures followed:

Artemia nauplii is one of the most important live feed in the rearing of shrimp larvae from mysis stage onwards and is a major source of contamination. . There is a possibility of contamination due to Artemia cyst outer covering and Artemia disinfection is not carried out by all the hatchery operators. Hatching of Artemia is influenced by the salinity, light intensity and aeration. Further when the nauplii are being fed the hatched outer covering could contaminate the larval rearing tanks.

Fig: 5: Hatching set up of *Artemia* cysts

Better Management Practices

- Disinfection of Artemia cysts should be done using hypochlorite solution. To disinfect the nauplii, the washed and de-watered nauplii are placed into a 15-20 litre bucket which is half filled with 10 litres of clean seawater.
- To this bucket, 125 ml of 50% hydrogen peroxide (H_2O_2) is added and the aeration turned on. The hydrogen peroxide will form bubbles and all of the unhatched cysts and debris will form foam on the surface of the water.
- The air is turned off after 5 minutes, the foam allowed to float and then the debris is removed using a small net.
- If decapsulated Artemia is used, the above steps can be avoided as it can reduce contamination to a great extent.
- To improve the hatching rate, instead of full-strength seawater, use 18-25 ppt seawater (which is made from seawater diluted with clean freshwater) at 28-30^{o}C.
- In order to reduce bacterial loading of the hatching tanks, either 60ppm of chloramines- T or 20ppm of suitable probiotics can be added to the tanks with the cysts.
- Switch on a light placed 30cm above centre of each tank and provide constant light (day and night).
- Artemia nauplii, which are active only should be collected by using their positively phototactic behaviour

11.Issue: Contamination through wet and live feed

Management measures followed:

Live feeds like polychaetes, squids, and mussels are known to be a major source of contamination and the hatchery operators generally collect these from different areas without confirming their quality. After collection, no measures are taken to reduce the level of contaminant in these live feeds

Better Management Practices

- To ensure that collection of live feeds is not from polluted sources.
- The live polychates and mollusc should be depurated to reduce the level of contamination before feeding.
- Screening after depuration should be done from time to time to ensure the quality of these live feed.
- SPF polychaetes can be procured to reduce any risk of infection creeping in through live wet feeds

Fig:6: Live polychaete worm feed

12. Issue: Food-safety issues

Management measures followed:

During the early stage of development of penaeid shrimp hatchery technology, use of antibiotics as a prophylactic measure was an integral part. Later when food safety issues cropped up with the presence of antibiotic residues in adult farm reared shrimps, the use of certain antibiotics has been banned in hatcheries. Antibiotics are used under the mistaken notion that they give better yield. Some hatcheries use banned antibiotics and other therapeutics causing environmental and health problems. Probiotics is gaining acceptance day by day, however proper method and the optimum dose for the strain of probiotics used are not given importance thus reducing probiotic effect and increasing the cost of production.

Better Management Practice:

- No antibiotics should be used for prophylactic purposes in larval rearing.
- Probiotic based seed production should be followed
- A good probiotic (high cell count ($>10^{8-9}$ cfu/g of multiple bacterial strains) should be used from zoea stage 1 throughout the larval and post-larval rearing stages and the concentration in water should be appropriate (aprox.10^{5-6} cfu/g)
- Based on tank size and dose rate appropriate amount of probiotic is weighed and added to a bucket with seawater and aerated for 1-24 hours. It is then filtered through a 100 micron (200 meshes) net. The solid material (bran carrier) should be discarded and the liquid filtrate added to the larval rearing tanks daily.

13. Issue: Ensure that discharge water from hatchery does not have any environment impact

Management measures followed:
Aquaculture activities contaminating the source water is one of the issues in all aquaculture ventures. The hatchery operation should ensure that discharge water from hatcheries meet all criteria for discharge water quality. Though ETP is present the proper waste water management practices are not followed.

Better Management Practices:

- All hatcheries should have a two level waste water treatment system for effective treatments.
- ETP procedure should be strictly followed with waste water being disinfected with proper disinfectant (like bleaching-60ppm) to ensure that it do not cause any adverse environmental impact

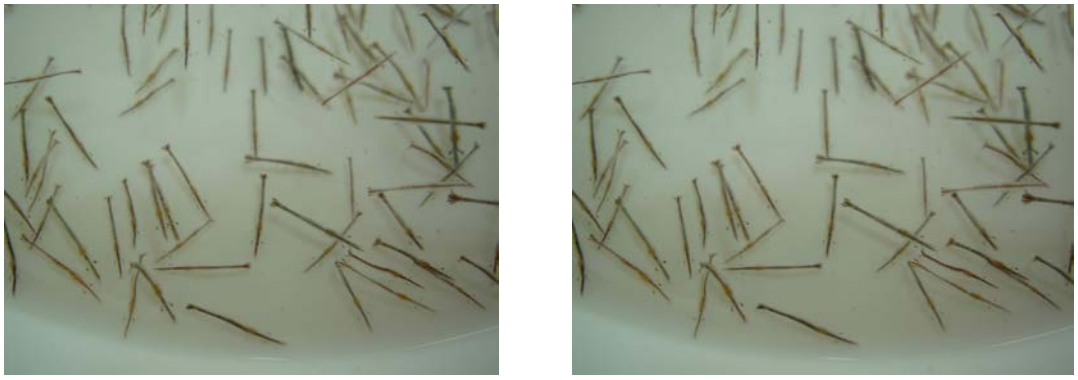

Fig. 7 Post Larvae

14. Issue: Ensure good quality shrimp seed through quality assessment

Management measures followed:
Though PCR testing becomes common with all the hatcheries, a holistic approach for total assessment of the shrimp seed quality is essential to ensure good quality seed.

Better Management Practices:

The holistic quality assessment is to be done at 3 levels

Level 1
Observation of animal and environment with examination based on gross features is to be performed on a routine basis. This should include: health examination of broodstock, sex determination, moult and ovarian development staging, removal of sick/moribund individuals, selection of nauplii by phototactic response, zoea/mysis stage feeding activity by observation of faecal strands, larval activity, postlarval health, activity and behaviour, stress.

Other aspects like good swimming activity, active phototaxis, faecal string, luminescence, stage homogeneity, intestinal contents are also important quality parameters to be judged. A preliminary

examination of PL in the tank is made to assess size distribution, benthic behaviour, swimming activity, feeding and colour. Then a sample is examined more closely:

Level 2

More detailed examination using light microscopy examination of larval and post larval quality and squash mounts, with without staining, and basic bacteriology of shrimp, feed and water should be included. For microscopic observation, 20-30 PL are to be randomly selected

Condition of the hepatopancreas and gut content, necrosis, deformities, epibiont fouling should also be part of the quality confirmation.

Level 3

Screening of broodstock, nauplli and postlarvae should be done by molecular techniques and immunodiagnostics (e.g. PCR). For *P. monodon* post larvae, WSSV is checked whereas for the *L. vannamei* WSSV, IHHNV along with other OIE listed virus should be screened.

In addition, vibrio examination should be done to check for potentially harmful *Vibrio* sp. bacteria in the PL. For this purpose, a random sample of 100 PL are taken from the tank, sterilized externally by dipping in 70% alcohol, washed, ground and then streaked with sterile wire loops, or 0.1 ml pipette. The PL pass if the number of green colonies is <60/plate and the number of yellow colonies is <80/plate on the TCBS plates, and if no luminescence is noted in the TSA media.

Fig. 8 Quality Shrimp Seed

15. Issue: Reducing stress during harvesting and transportation

The PL should be acclimated in the hatchery to the salinity expected in the on-growing farms. This is to reduce the stress on stocking in the ponds, a critical point in the process which can lead to high mortalities if not done smoothly.

Better Management Practice

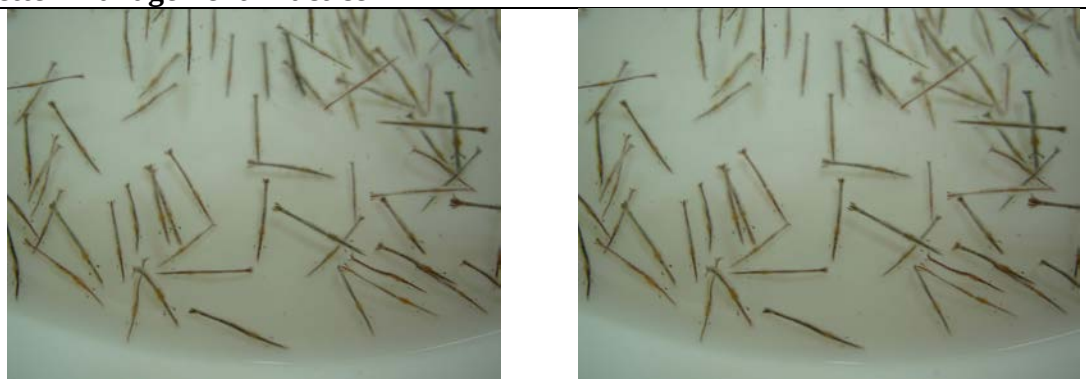

Fig:9. Post Larvae

General test

Good quality post larvae should be selected through observation and microscopic, molecular and stress testing. The 6 criteria used are scored using a standard score and are: (i) hepatopancreas (ii) gut condition (iii) fouling (iv) Deformity (v) muscle : gut ratio (vi) MBV . For the stress test the PL must be at least 10 mm long (>PL8-10) withstand these tests, and it is ideal to test it as close to stocking date as possible – i.e. PL 12-15 at >12mm total length.

Stress test

Stress testing is done using two stress factors of salinity and formalin.

Survival (%) = (No. active PL/total PL In beaker)* 100

Scoring: the PL batch passes if the survival >75% and fails if below score.

PCR Testing

PCR Testing for WSSV can help reduce the risk of crop loss due to this disease and should therefore be checked on each batch of PL stocked.

PL Harvest and Transportation

Transportation should be stress free and density should be maintained as per the duration of transportation. Salinity adjustment can be done by adding freshwater to achieve a salinity change of <3ppt/hour from 30-20 ppt, but should be reduced to <1ppt/hour from 20-10 ppt and <0.5ppt/hour from 10-5 ppt. Such salinity adjustments should commence only when the PL are older than PL 10 (preferably 2-3 days before harvest), when their gills are fully developed (looks like Christmas tree) and they are able to tolerate such rapid salinity changes.

Conclusion: Best Management Practices (BMPs) in the hatcheries are essential for producing quality seed. Unless quality seed is ensured by adopting BMPs, the whole production-consumption system in the value chain will be affected. Though many attempts have been made to develop and disseminate BMPs, due to location specific problems, these have not been widely implemented. There is a general lack of qualified staff in the hatcheries and awareness on the technical details for implementing these BMPs. Ultimately, what we require is a twin approach of developing a manual with specific details for all the 15 BMPs identified in this publication and a framework for analysing location specific problems that would require additional measures to ensure that these BMPs are implemented. The present publication is aimed at highlighting such location specific issues that need to be examined and drawing attention to specific bottlenecks in producing quality seed.

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