Food and Agriculture Organization of the United Nations

Manual on Propagation

# of Large Cardamom

MANUAL ON PROPAGATION OF LARGE CARDAMOM IN BHUTAN

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INTRODUCTION

Large cardamom (*Ammomum subulatum*) is an extremely important spice crop for Bhutan. Around 17,000 farmers in Bhutan are engaged in its production with earnings of US$ 500-1200 per farmer. Bhutan is generating a foreign exchange of US $13 million as of 2014 (Mehta et al., 2018.)

It is grown in lower altitudes, receiving around 1,700-2,500 mm rainfall per annum, preferably under hill shade and moist conditions. In the higher altitudes, farmers grow the crop in high rainfall conditions (2,500-4,000 mm per annum). The crop exhibits slow growth during the dry season and can withstand up to 1°C. The plant is highly susceptible to frostbite. According to a cardamom grower "Low-intensity snowfall does not necessarily hamper the production potential but prolonged exposure to sunlight will dry out the leaves". Leaf drying in large cardamom is a burning problem in Bhutan.

Review of several pieces of literature shows that Bhutan's climate is extremely suitable for its cultivation and there is sufficient possibility of its expansion in terms of acreage and volume.

At present, there are few government farms in Gelephu and Tsirang; and private nurseries that are producing saplings by seeds. They are mostly raising Bharlange variety for production. Farmers are mostly propagating saplings by asexual method. This has further aggravated cardamom industry in Bhutan because of disease transmission. Disease incidence has aggravated large cardamom industry at Bhutan by reducing productive life, becoming a source of disease spread, and thereby reducing enthusiasm of the stakeholders engaged in the value chain of large cardamom. We have to discourage these practices.

The supply of high quality, and disease-free saplings is one of the basic components for the modernization of this industry. We have to give

extension booklets to farmers while selling large cardamom saplings. This booklet should possess all relevant information on cardamom so that farmers can identify appropriate variety suitable to their condition and other planting details. Government authorities should avail them to all sapling production facilities of Bhutan.

This manual will give a glimpse about the various methods of propagation techniques, their advantages &disadvantages, equipment, materials and effort requirements. Furthermore, it will help farmers for the establishment of the nursery and high quality, disease-free saplings. We hope that it will be useful to those who are engaged in large cardamom business in Bhutan.

LARGE CARDAMOM PROPAGATION METHODS

Large cardamom should be propagated through three methods vis-a-vis sexual, asexual and micro propagation techniques.

SEXUAL METHOD

The sexual propagation of large cardamom is done through high yielding seeds. This method enables the production of seedlings in large numbers. It prevents transmission of viral diseases through seeds so the seedlings produced are free from viral diseases like “Chirkey” and “Foorkey”; however, adequate care needs to be taken to protect the nursery from fresh infection from nearby plantations.

Merits

- Seedlings are long lived compared to suckers detached from plantations.
- Seedlings produced through this method are free from viral diseases.

Demerits

- Costly compared to vegetative propagations.
- Variety may not be true to the type because it might be cross-

pollinated with wild or other variety.

- This method may allow transmission of fungal diseases.
- Seedlings will be costlier than those produced from asexual propagation
- Following steps need to follow for sexual propagation

PRIMARY NURSERY

Site selection for Primary Nursery

a. Select site with sufficient sunlight illumination and sufficient water for irrigation.
b. The site must be free from snow or frost; south faced nurseries can get sufficient light and heat.
c. The Site should be at least 500 meters away from the main plantation to avoid transmission of pest and diseases.
d. There should be no water logging
e. There should be no trees around because it will hamper sunlight illumination.
f. Loamy soil with abundant amount of organic matter is preferred.
g. Road access will help for input, output dispatch, and reduce cost.

Variety Selection

a. Large cardamom is highly cross-pollinated. So, extra attention in selecting the bush must be given. Presence of other variety, wild relatives of cardamom can deteriorate the seed quality. It will not exhibit real varietal characteristics.
b. Select varieties depending upon the yield potential, tolerance to pest and diseases and the altitude.
c. Disease-resistant varieties are Zongu-Golse, Seremna, Bharlange, etc.

Plant Selection

a. Select plants, which are free from disease, well performing and true to the type.
b. The selected variety should a have high record in production potentials in the past recorded years.
c. Keep high performing plants as a mother stock as mentioned in the variety maintenance section of varietal identification manual.

Capsule Selection

a. Collect the capsules from high yielding and well-maintained plantation.
b. Select capsules that are well matured, big, & disease free.
c. Extract it from spikes that look healthy and productive.
d. Take the bundle of capsules and let them piled to warm for two to three days to make easy to detach.
e. Select bigger sized, well matured, capsules from the basal and middle portion of the spikes.
f. Segregate capsule in terms of size and weight. Use only weighty and bigger sized capsules.

Seed Selection

a. Select capsules from well performing, healthy bushes, which are true to the type.
b. The capsules in the tip area are small and not recommended to use for propagation purpose.
c. Select well-matured capsules having black seeds inside. Separate light seeds from weighty ones. Use only weighty seeds.

Seed extraction

a. Cardamom seeds remain covered with sticky mucilage. It will be necessary to remove mucilage by rubbing with sand and ash mixture.
b. Repeat the process until there is no mucilage attached to the seeds.

c. Rubbing the seeds with jute bags and with sand is another way of seed extraction.
d. Wash the extracted seeds with clean water and dry under shade for 3 to 4 days. The seed obtained after drying can sow immediately or stored. It should be stored in a cool and dry place.
e. The germination percentage of the seed extracted through this process is 30 to 50 percent.
f. Steps for seed extraction as per the Cardamom Research Center, Ilam, Nepal, 2015
g. The cardamom seed after extraction from the capsule is mixed with one kilogram sand and half kilogram ash and leg rubbing is practiced rigorously.
h. This procedure must be repeated for five times.
i. The seed is then kept inside muslin cloth.
j. The pit is made atone and half feet depth where a half foot is filled with sand.
k. The seed pack is placed on the sand.
l. The pack is again covered with sand of half feet level.
m. Good result can be obtained if this process is practiced through the last week of January to the first week of February.
n. The seed is taken from the pit after one to one and a half months for sowing in nurseries.
o. The germination will begin after one month of sowing.
p. The germination percentage was around 65.
q. The seeds which fail to germinate will decay in the soil.

Removal of mucilage using acid

a. As per the recommendation of Indian Cardamom Research Institute in Gangtok, Sikkim, cardamom seeds are dipped in 25% nitric acid (25 ml nitric acid and 75 ml water) for 10 minutes.
b. The seeds are then thoroughly washed in running water for removing the acid.
c. Germination of seed following this method of seed treatment starts

within four to five months of sowing. Germination depends upon the temperature and humidity of the nursery.

Nursery soil sterilizations

a. Soil sterilization can be practiced to minimize pest attack by covering nursery beds with transparent plastic for a month or more before going for the nursery.
b. Manures can also be sterilized using the same procedure.
c. The best time of soil sterilization is before September.

Manure applications in nursery

a. Adequate manure should be applied on the well prepared nursery beds.
b. Take care of soil laboratory recommendations.
c. Two square meter land should 20 to 25 kg well rotten compost, one kilogram ash and 10 to 15 kg sand if the land is clayey. These ingredients should be nicely incorporated.
d. The seedbeds must be free from the clods, weeds and fine-grained.
e. Mulching with black plastic are also practiced for fast germination.
f. Time of application: Before September.

Nursery bed preparation

a. Nursery bed of one-meter width and length according to the land and convenience should be prepared.
b. The seedbeds should be raised 25 to 30 cm from the ground.
c. There should be at least a **one-meter** gap in between two nursery beds.

Seed sowing

a. Seeds can be sown immediately after seed extraction.
b. Best time of seed sowing is through last week of September to mid-February.
c. One gram of treated/extracted large cardamom seeds is recommended for a square meter of a nursery area.

d. Seeds are sown at 2 cm depth maintaining 2 cm row to row distance.
e. The sown seeds are thinly covered with fine-grained soil from above.

Mulching

Mulching helps to retain soil moisture and reduces weed pressure in the nursery.

a. After the seeds are sown, the beds should be mulched using straw or plastic sheets.
b. If straw is used as mulching material, the thickness should be maintained to 3 to 4 cm.

Germination

a. The time taken for seed germination of large cardamom depends on the method of seed treatment used. It takes 3 months to 12 months.
b. The germination percent of large cardamom is reported to be 30 to 65 percent.
c. Mulching should be removed as soon as germination starts so as to facilitate photosynthesis by the seedlings.

Shade Management

a. Shade is necessary to protect the plantlets from the direct sunlight, hail stone, heavy rain, frost and snow.
b. 50% shade should be provided using shade nets or bamboos roof.
c. It will also protect them from insect bites and diseases incidence.
d. Provide different shade net in the nursery to different varieties of large cardamom to maintain true to the types in the same nursery and for easy identification during transplanting.

Irrigation

a. Irrigation is done 3 to 4 times in 3 to 4 days interval depending upon the soil moisture content and occurrence of rain.
b. Overhead sprinkler irrigation or drip irrigation system can be installed in the nursery for efficient watering and to save labor. Do not allow water logging at any cost.

SECONDARY NURSERY

The nursery where 3-4 leaf seedlings from the primary nursery are transplanted for further multiplication through proper care is called secondary nursery.

1. A secondary nursery is prepared just like the primary nursery.
2. The plants from the primary nursery are weeded, uprooted and worked.
3. The week and inferior saplings from the primary nursery are removed and discarded.
4. Apical portion above the emerging shoot and roots except for 5 to 7 cm roots are cut before transplanting for hardening and establishment.
5. Three plants are planted in a three adjacent points in triangular style maintaining line to line distant of 40 cm and plant-to-plant distance of 30 cm depending upon the fertility of the soil and robustness of the variety.
6. After transplanting, soil at the base are pressed using hand for better establishment.
7. Mulch the entire nursery using straw to control weed and retain soil moisture.
8. The entire nursery should be maintained under overhead shade preferably black agro-shade nets.
9. A layer of well-decomposed cattle manure is applied and incorporated in the nursery soil.
10. Other management practices are similar to those of the primary nursery.

Other tips for secondary nursery

- Keep the seedling well managed in the secondary nursery for 10 to 12 months.
- At the end of 10 to 12 months, each seedling is expected to attain45 to 60 cm in height with5 to 10 tillers.
- Old mother plant of primary nursery can be removed and the young offsprings are maintained.
- At the time of selling, count one tiller along with one healthy vegetative bud as one seedling.

Packaging of the seedlings

- Seedlings are packed in jute bag wrapping with sphagnum moss after soaking the moss in water.
- In a bundle, we can keep around 70- 100 saplings pack. Generally, 100 saplings per pack are used in nurseries.
- It should be convenient to transport.

ASEXUAL METHOD

Asexual propagation refers to the removing of stem sections or root tissue from the parent or donor plant, treating this tissue with plant growth regulators, and then inducing adventitious root or shoot formation under controlled/open environmental conditions. (Susan R et. al., 1992). In large cardamom, asexual propagation is done through its suckers and rhizome buds (tissue culture).

Merits of Asexual Method of Propagations

- They are easy to establish orchards and cheaper than other methods.
- It starts fruiting from the second year of planting.
- Superior high yielding mother bush originated saplings will give better performance.
- Large number of saplings with characteristics true to its mother plant (desired varieties) can be produced.
- Faster orchard establishment compared to sexual methods (3 Years).

Demerits of asexual propagations

- There will be transfer of diseases at newly established orchards if the mother plant is diseased.
- It will rapidly multiply the disease.
- Difficult to transport.
- It is not advised to take saplings from older mother stocks less than 15 years because loss of vigor can make new orchard weak and susceptible to pests
- Require heavy manure application.

Points for consideration

- Choose only healthy, well performing bushes only.
- Mother plant's hygiene is very important in such asexual propagation.
- It is believed that the incidence of fungal, viral diseases of cardamom will be high in asexual propagation.
- The knife and cutting instrument must be sterilized using hot water before and after use. Use equipment after they cool down.

Maintenance of mother stock or variety repository

The Repository is the mother stock of Large Cardamom of the highest quality. Variety repository is kept inside a net with high care. The planting materials (suckers, rhizomes, root, and one plant) are taken from this mother stock.

Purpose

To obtain high quality, disease-free planting material for propagation purposes.

Facilities required for the science-based repository

1. Tunnel with 50 per cent shade net
2. Irrigation facilities (drip or sprinkler irrigation)
3. Adequate manure application based on Soil test result

4. The suitable place for the repository established will be above 700 masl
5. Frequent disease test

How do we establish the repository from the scratch?

- Select disease free and high yielding true to type variety from the field
- Laboratory test for diseases and genetic deformities
- Keep the selected varieties in the repository.

Further maintenance can be done by following approaches;

- High yielding, true to the type mother stocks of different varieties should be maintained.
- They should be maintained under intensive care and management.
- Each variety should be maintained in separate cabins.
- They must be kept mostly under agro-shade nets.
- Regular spray of fungicides like blitox 50 @ 2ml/litre of water in 15 days interval periods should be practiced.
- Manure application should be practiced @ 10 kg/bush twice or thrice a year.
- Irrigation from showers should be practiced as needed.
- Removal dead, older leaves must be done.
- Limited number of people should be let inside, but only after putting on aprons.
- Plant parts from these bushes should be used for propagation.

PROPAGATION THROUGH SUCKERS

The easiest way of large cardamom propagation is through separation from its mother plant directly from the plantation. The farmers practice it widely as it is cheaper compared to other propagation methods. However, there is a high chance of both viral and fungal disease transmission in this method. In order to minimize the risk of disease transmission, a separate healthy sucker multiplication nursery should be maintained under proper management.

Establishment of sucker multiplication nursery
Selection of planting materials for nursery establishment

- High yielding, disease-free plantation of desired variety should be selected.
- The plantation should have a high yield record (depending upon the variety) for the past 3 years.
- A healthy and matured tiller with two immature tillers or vegetative buds showing desired characteristics of a chosen variety should be selected for planting in the sucker multiplication nursery.

Site of sucker multiplication nursery

- The site for nursery and main plantation should be at least 500 meters to avoid transmission of pests and diseases in the nursery.
- The nursery should be maintained either under the shade of forest or under shade pandals with 50% shade using agro shade nets.

Preparation of trenches

- Trenches of size 30 cm width and 30 cm depth with a convenient length depending upon the land are prepared across the slope of the field.
- Maintain 30 cm distance between the trenches.
- The well-decomposed cattle manure or compost should be filled into the trenches and mixed thoroughly with the topsoil.

Planting of suckers

- The suckers with a mature tiller with two immature tillers or buds are planted at 30 cm apart in the trenches.
- The bets recommended time of the plantation is in the month of May and June.
- After plantation, the plant base is mulched with dried forest leaves.
- The multiplication rate is about 1:8 in one year.

Management and maintenance of the nursery

- Monitor consistently and rough out old, off-types, diseased plant from the sucker multiplication nursery.
- Mulch the nursery using dry leaves/grasses.
- Time to time weeding is done depending upon the weed pressure.
- Irrigation should be done during the dry season usually from November to March depending upon the soil moisture content.
- Irrigate the bush before uprooting saplings for sale.
- Cut one-third of the apical and root parts to minimize transpiration and ease transportation.
- Keep one-foot long pseudo stem only to prevent pseudo stem bending while handling.

Packaging of sapling for dispatch

- Pack the saplings counting one plant with one bud, rhizome and roots.
- Farmers can detach them from the whole bundle at the time of planting.
- Treat them with copper-oxychloride solution @ 2ml/liters of water concentration.
- The uprooted plants should be packed with sphagnum moss wrapping with jute bag.
- Apply water in the bundle from time to time.
- Jute bags and sphagnum moss are used in wrapping the sapling.
- It will help to preserve water.

TISSUE CULTURE

Tissue culture is the method of 'in vitro' culture of plant or animal cells, tissue or organ – on nutrient medium under aseptic conditions usually in a glass container. Tissue culture is sometimes referred to as 'sterile culture' or 'in vitro' culture. By this technique, living cells can be maintained outside the body of the organism for a considerable period.

According to Black Well, 1973, tissue culture is referred to as any multi-cellular culture with protoplasmic continuity between cells and growing on a solid medium or attached to a substratum and nourished by a liquid medium.

Toti potency– Each plant piece (cell, tissue or organ) is capable of growing a whole plant, which will be identical to the mother plant.

Plant tissue culture is a collection of techniques used to maintain and grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of a known composition.

Importance of plant tissue culture in large cardamom

The process of propagation facilitates the production of a large number of diseases free quality plantlets independent of any seasons. In fact, plant tissue culture has several applications extending from mass propagation of the plants by micro propagation, or in vitro culture; generation of quality planting material, production of phyto-chemicals and high-value pharmaceutical cosmetics and food additives (CSIR,2016). The apical growing part or meristematic tissue is considered to be free from viruses. Its rapid multiplication at aseptic condition within a laboratory is practiced. But it must be tested for all kinds of diseases before culture.

Thus, this technique can be applied in large cardamom propagation to produce a large number of disease free seedlings with the desired characteristics in a short span of time.

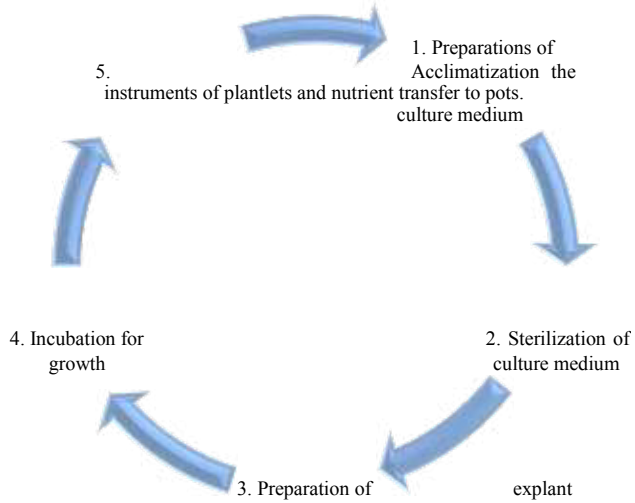

Figure 1: Steps in Tissue Culture

Preparation of the instruments and nutrient culture medium
Apparatus required

1. Forceps
2. Scissors
3. Blade holders
4. Disposable but sterilized surgical
5. Petri plates with two filter papers tips.
6. Pipette 1000ml
7. 50 ml test tube
8. 500 ml beakers
9. 1000 ml bottle with crew caps
10. Three 350 ml early mire flasks blades or sharp carpels
11. A box containing disposable pipette
12. Pipettes

These apparatuses need a thorough cleaning. After cleaning them, they are wrapped in newspaper and transferred for autoclaving.

1. All culture vessels must go for washing before going for tissue culture. These are then sterilized. These are autoclaved for one hour.
2. Glass and plastic-wares that are to be used for culture should be washed completely and autoclaved for one hour.
3. Then they are immersed in chromic acid for 16 hours.
4. Then glass and plastic wares are first rinsed with water to remove chromic acids immersed in detergent water and cleaned by hard deluge process.
5. Then the glass and plastic wares are then immersed in clean water. And then detergents are rinsed off.
6. Finally, the glass and plastic ware washed under running tap water kept in clean trays and put in an oven ready maintained at 60 -80 degree Celsius for drying. The dried glass wares are now for use.

Sterilization of culture medium

1. Next important process is preparation of culture medium which is to be in the process of micro-propagation. A medium for plant tissue culture will comprise of a group of macro element, and micro elements vitamins, amino acids, and more importantly sucrose which serves as a source of carbon which is the carbohydrate for the plants. The amounts of salts taken have to precisely weighed as per reported for relations and these have to be dissolved in de-ionized water.
2. Care has to be taken to avoid precipitation of the salts as it prevents sufficient availability of salts to the plants.
3. Salt of higher solubility are first dissolved.
4. Adjustment of the pH of the medium. Generally, plants can take up nutrients and the pH ranging from 5.6 – 5.8
5. After the medium attains the desired pH, it is ready to be poured into 250 ml early mire flask to 50 ml test tube as 100 ml or 20 ml flask.

6. Semi solid medium has to be prepared. The method follows weigh to be 0.75 – 0.8 g of agaragar. This amount has to be optimized to provide optimum moisture to the culture.
7. Pouring of medium, 100 ml of medium is carefully poured into each 250 ml early mire flasks and plugged using cotton plugs made of nonabsorbent cotton wool covered with muslin cloth and tied at the top allowing easy holding of the plug while inoculation.
8. Next step is sterilization of the culture medium by autoclaving. An autoclave is a device used to sterilize equipment and supplies by subjecting them to high pressure saturated stem. The media is prepared and the different instruments are made ready for use which are autoclaved for 20 minutes at high temperature i.e. 120 ^{0} C and pressure 15 Pascals.
9. These parameters are taken to sterilize the instruments and the media without degrading its composition such as sucrose etc.

Preparation of explants

Figure 2: Lower bud is for pseudo-stem and upper bud is for flower initiation

Meristematic tissue from such place can be taken as explants.

1. Different kinds of explants such as leaves, petals, buds, ovaries, seeds anthers, and noggle segments can be used for a plant tissue culture because each and every cell of the plant is capable of giving rise to new individual. However, care must be taken to collect these explants from disease free healthy and actively growing plants. It is preferable to have some meristematic areas of high cell activity.
2. Immediately after collection, explants are placed in clean water to avoid entrance of air bubbles, microbes and contaminants from the cut or exposed parts and to avoid browning due to phenolic oxidation.
3. These explants are then brought into the laboratory for surface sterilization. For this, explants are first cut into smaller size using scissors and then placed in a Petri dish containing clean water.
4. The surface of the explant is then brushed to clean with the mild detergents such as twin 20, as a wetting agent with several hair brushes. After cleaning the surface, the explants are picked up and dropped into the glass of a plastic vessel containing a mild solution of fungicide or an antibiotic.
5. The explants are then swelled for few minutes and rinsed several times with clean and sterilized de-ionized water.
6. Finally, explants are immersed in sterilized, de-ionized water and taken into an inoculation chamber.

Inoculation of explants

1. Now our explants are ready for the next process of inoculation.
2. Prior to entry into the inoculation chamber, through the double door, it is important to wear a clean cotton lab coat and take an air shower.
3. Inoculation means transferring of plants specimen into the media under aseptic conditions.

Preparation of laminar hood

1. The laminar hood is special equipment for inoculation.
2. Shut UV light can kill all the microorganisms by the shutter of the laminar hood. Switch on the UV light for about 40 minutes.
3. It is highly dangerous for human eyes and skin. Therefore, the shutter has a black sheet and is not open until the UV light is switched off.
4. After 30 minutes, switching off of the UV light, switch on the chamber light. Open the shutter and then switch on the airflow.
5. Prior to operating the laminar hood, it is essential to wear a clean and sterilized face mask and cap.
6. The panel of the laminar that faces us has an efficiency particle air filter.
7. The explants are washed with 70 percent ethanol for a few seconds and then treated with the mild mercuric chlorides solution for a few minutes.
8. A general thumb rule is to use a mild solution for a longer duration rather than a strong solution of short duration.

Inoculation of explants.

1. After the sterilizing agent treatment, the solution is decanted into the waste beaker.
2. And the explants are washed several times with sterile de-ionized water to remove all traces of mercuric chloride.
3. All the apparatus are dipped in the 70 percent ethanol flamed using the light of the spirit lamp allowed to cool.
4. After cooling, the explants are carefully picked by the forceps and placed in the Petri plates with filter paper so that all the excess water content will be absorbed.
5. The Surface of the explants which were exposed to mercuric chloride, are removed by the help of a surgical blade.
6. Each nodule segment is carefully inserted into the medium and contained in the test tube and care is taken to avoid explant from

touching the rim of the flask which is again flamed and after that cap is put back at the mouth to seal tightly.

7. Finally, the name of the plant, medium, and date of inoculation is labelled onto the surface of the test tube.

Incubation for growth

1. The cultures are now ready to be incubated in the culture lab at optimum conditions of 16 hours light alternating with 8 hours darkness.
2. Twenty-five to twenty-seven ^{0} C temperature and 40 percent relative humidity and it is monitored timely.
3. After a few days, which may range from 5 to 15 days, all cultures with either bacterial or fungal contaminants are removed, and the healthy cultures are allowed to grow further. After a period, one sample with the large number of shoots is immersed in a single flask.
4. Once the shoots are grown certain appreciable height of about 3-4 cm they are rooted.
5. Generally, auxins are used in the culture medium to induce rooting in each of these shoots. Once the shoots are rooted, they have to be hardened for acclimatization in an open environment.

Acclimatization of plantlets and transfer to pots.

1. Now the tissue cultured raised plantlets (TCPs) are transferred to a green house or outdoor conditions. They are subjected to different types of shocks like temperate, humidity, nutrition, carbon dioxide, and air flow shock.
2. The green house and fields have substantially low humidity. Higher light intensity and septic environments and are therefore, stressful to the TCPs because they have grown out of the comfort zone of in vitro conditions.
3. After rooting and growth of plantlets up to 3-4 inches shift these to other potting mixture containing garden soil, sand, and well-

decomposed farmyard manures in ratio of 1:1:1.

4. Other mediums like soil rite, vermiculite, perlite, and coco pit can also be used for preparation of the mixture.
5. After 45 – 60 days, these acclimatized plantlets can be shifted to the outdoor conditions. These plants are now ready for the harsh conditions effectively with a very low mortality rate.

Figure 3: Micro propagation of large cardamom

PROTOCOL FOR LARGE CARDAMOM TISSUE CULTURE

In vitro micro-propagation is one of the best alternative methods of propagation for rapid clonal mass propagation for good and healthy high yielding plant with minimum diseases. MS fortified with BAP (1.0 mg/L) and IBA (1.0 mg/L) is the best media for root and shoot induction. This protocol is effective for mass production and multiplication of large cardamom (Poudel et al, 2018).

Hardening of Tissue cultured plants

a. The newly emerged plants are extremely fragile therefore are placed in room temperature outside the incubation chamber for about 24 – 30 hours.

b. After room temperature maturing, these plantlets should go to glass house or green net house where the entry of the insects (Aphid) will not be possible.
c. The media of the culture will be sand, vermin-compost, and forest soil mixture in 2:0.5:1 ratio.
d. This media is then sterilized by the formalin 1% solution. (39% Formaldehyde is available in the market).
e. The person who will take care of the saplings should wear sterilized aprons.
f. Lime water should be there at the entrance of the shade house/ net house/ glass house.
g. The person taking care of the saplings should not smoke.
h. For organic sapling production, Nibicidin or Marglobe at the rate of 3 to 5 ml per liter of water is used for insect control. It is a neem-based insecticide.
i. For organic production, one-month solarization inside the white plastic will kill the pathogens.
j. The plantlets are planted in benches 20x10 cm apart.
k. The media should be fertilized with nitrogen, phosphorus, and potash at the rate of 200:200:100 kg per hector.
l. Half kg mixture of micro nutrients should also be added per ha.
m. The plantlets and media should get a regular spray of 0.02 percent bavistin solution.
n. To increase humidity, they are covered with white plastics for 4 – 6 days but excess temperature will kill the saplings. Hence, proper care should be taken.
o. The water that is used in the irrigation of plantlets should be treated by ultraviolet rays filter for water sterilization.
p. The insects can be removed by killing them with electric netted bats.

Sale of saplings for plantation

The plantlets are wrapped in sphagnum moss and tied in jute bags for transport to the planting field. It should have around 8 to 9 leaves and height of one foot. Pseudomonas fluorescens is widely used as a bio-agent for disease management in large cardamom in Sikkim. The mass multiplication technique and field application schedules were worked out at Indian Cardamom Research Institute, Regional Station, Spices Board, Tadong, Gangtok, and Sikkim for large cardamom cultivation. The bio-agents, by virtue of their properties such as quick growth, fast multiplication capacity, antagonism to disease causal organism, hyper-parasitism and competition with other microbes, etc., suppress the pathogenic fungi and promote growth and protect the plants from various soil-borne fungal diseases of large cardamom. These plants are then taken to the fields for plantation.

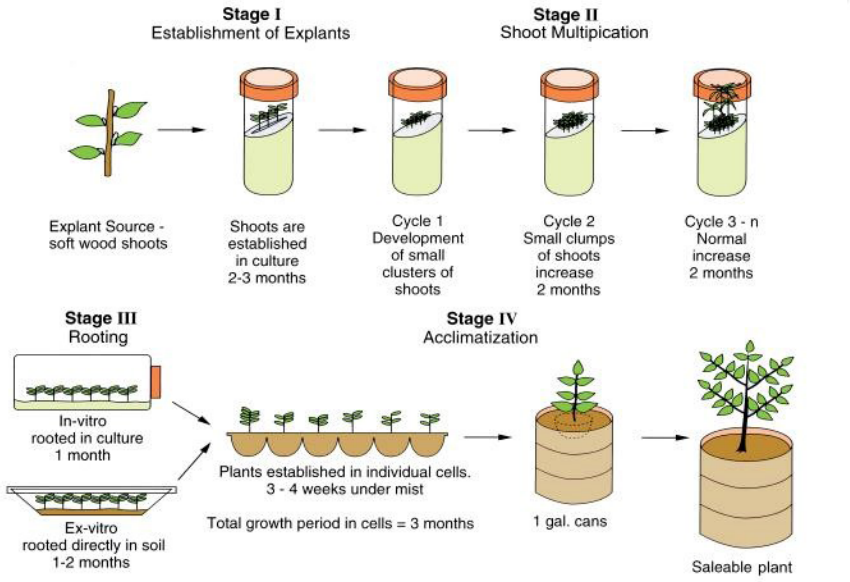

Figure 4: Various process of sapling production by Tissue Culture

NURSERY PEST AND DISEASE MANAGEMENT

Insects

Some of the most common insect pests of large cardamom nursery are White grub, Thrips, Aphid and Sand Stem borer, but stem borer insects are not serious in nursery environment.

Thrips - Biology

Egg: The adult female lays eggs on the leaf or fruit surface. Just before hatching the egg blisters. The eggs are white and banana-shaped and are inserted singly in plant tissue.

Larva: The early larval stage is whitish with red eyes. Larvae become yellowish after feeding. Mature larvae average about 1 mm in length. There are two larval instars and then it moults to the pre-pupal stage, which is light yellow with red eyes and short wing pads.

Pupa: The pupal stage is slightly larger, with longer wing pads and larger eyes. It is yellowish and then darkens with age. The antennae are bent backward over the head in the pupal stage. The prepupal and pupal stages do not feed.

Adult:

- As it matures into an adult, the greenhouse thrips' head and thorax darken to black while the abdomen changes from yellow, yellow-red, brown, and black.
- Cool temperatures retard the color changes.
- The legs remain a light yellow, and the antenna has eight segments.
- The greenhouse thrips is parthenogenic, in that it reproduces without mating, and males are seldom seen.
- It is a poor flier and remains in the shaded areas on the plant almost all the time.

Damage symptoms

- This pest feeds primarily on the foliage of ornamental plants.
- It attacks the lower surface first and, as feeding progresses and the population increases, the thrips move to the upper surface.
- The leaves become discoloured and develop distortion between the lateral veins.
- Severely damaged leaves turn yellow.

Figure 6: Thrips

Figure 5: Different stages of Thrips

Aphid - Biology

Egg: Eggs are kidney shaped and are laid singly in the tender parts of the leaf sheath, racemes.

Nymph: Nymphs are tiny, slender, fragile and straw yellow in color.

Adult: Adult Aphids are minute, dark greyish brown, 1.25 to 1.5 mm long and with fringed wings.

Damage symptoms

- Panicles become stunted.
- Shedding of flowers and immature capsules, thus reducing the total number of capsules formed.
- Infestation causes formation of corky encrustations on the capsule resulting in their malformed and shriveled condition.
- Such pods lack their fine aroma and the seeds within are also poorly developed.

Natural enemies of aphid

Parasitoids: Aphidius colemani, Aphelinus spp.

Predators: Lacewing, ladybird beetle, spider, syrphid larva.

Figure 7: Banana Aphid

Figure 8: Peach Aphid

Stem Borer

It is a serious infestation problem in the fields because in the outside environments stems of large cardamom are developed. In the nursery, the stem is not that developed. It is recommended to rough them out in case of infestation. The net do not allow its entrance from outside.

Figure 9: Stem Borer

Figure 10: Adult Moth

White Grub - Biology

Eggs: Adult beetles emerge by March-April and lay their eggs in the soil. The eggs are soft, ellipsoid, off-white, and about 1 mm long on the longest axis.

Grubs: The larvae are fat, whitish, or cream-colored grubs, and generally about 38 mm long when fully grown. The newly hatched grubs emerge during June-August and continue to develop up to October/November. During this period, the feeding grubs are found in the top 6 inches of the soil but may move deeper when the soil is very dry. The larvae feed on plant roots and organic matter in the soil.

Adults: The adults are typical chafer beetles, mostly brown, and 19-20 mm long.

Damage symptoms

Affected plants show yellowing of the foliage, scorching of leaves, defoliation, and dieback. Inspection of the root system will reveal that the roots have been chewed off leaving calloused stumps. *H. disparilis* also feeds on the plants at soil level, causing damage to the stem followed by death of the plants.

Natural enemies of white grub

Nematode: Heterorabditis nematode.

Treatment

- White grub can be controlled by using Metarhizium @ 1 gm. per square meter.
- Damping off of the seedlings can be controlled by applying a mild solution of carbendazim @ 0.5 ml/l of water.

- The major insects that harm seedlings should be removed or killed (Chloropiriphus 2 gram).
- Neem based organic insecticides like nimbicidine and marglobe can be mixed to 3 ml/litre of water and applied to the seedlings.
- In case of viral diseases, rough out the infected plant and destroy it.

Figure 9: White Grub

Caterpillars - Biology

Egg: 300 – 800 eggs are laid on the under surface of leaves of shade trees. The eggperiod is 13 – 20 days.

Larva: Larva is hairy and has a darkgrey body, pale brown head. Larva undergoes 10 instars in 5 months.

Pupa: Pupate is buried in soil at a depth of 2 – 2.5 inch; pupa is cocooned; pupal period is 7– 8 months.

Adult: The adult is a large moth measuring 70 -80 mm, ochrus in color with post medial lines on the wings.

Damage symptoms

- The caterpillars congregate on the trunks of shade trees and then descend to the cardamom plants.
- They voraciously feed on the leaves of cardamom plants, defoliating within a short time.

Figure 10: Adult

Figure 11: Cardamom caterpillar

DISEASES

Damping off, Chirkey and Foorkey, Blight, Wilt, Leaf streak are common diseases.

Chirkey Disease Symptoms

- The symptom is more prominent on young emerging leaves where discrete pale green to yellow longitudinal strips running parallel to each other can be seen.
- Mosaic appearance on the tender leaves with pale streaks can be seen.
- Growth, flowering and yield of affected plants gradually decline and ultimately they perish.

Figure 12: Chirkey Symptom

Pathogen and Transmission

- The disease is transmitted by the insect vector. (Corn aphid*Rophalosiphum maidis*)
- It also spreads by planting infected suckers.
- This disease spreads due to transportation of infected suckers from one to another.
- The disease is also transmitted mechanically through knives used for harvesting.

Foorkey Disease Symptoms

- Numerous small tillers appear at the base of the affected plants, which become stunted and fail to give any yield.
- The leaves shrink in size; become lightly curled and pale green in color. Sometimes slightly broadened leaves resembling that of betel leaf are also seen.
- Inflorescence becomes stunted producing no flowers and fruits.

Pathogen and transmission

- The disease is caused by the virus and is transmitted mechanically through sap by the insect vectors such as banana black aphid, *Pentalonia nigronervosa* and *Micromyzus kalimpongensis.*
- It is also transmitted through planting by use of infected propagation materials.

Control measures

Due to Foorkey being a viral disease, the affected plants cannot be fully cured but the losses can be minimized by adopting appropriate management practices:

- Monitor the plantation regularly, particularly more so during rainy season sand carefully identify the diseased plants.
- Adopt a regular roughing process of

Figure 13: Foorkey Symptom

infected plants as soon as symptoms appear (uproot and destroy affected plants).

- Mass uprooting and burning of infected plants at the village/ community level could be taken up for eradication of the disease.
- Use seedlings produced in certified nurseries. Never collect planting material from an infected plantation or apparently healthy plants from severely infected gardens.
- Propagation through suckers is recommended only through certified multiplication nurseries.
- Establish nurseries about 500 m away from the main plantation in order to avoid aphid colonization
- Maintain clean clumps by removing old tillers with loosened leaf sheath so that aphid will not colonize.
- Use of clean field equipment is highly recommended.

Damping off Disease Symptoms

Seedling gets infected with black spots in the area nearby the soil and the seedling base.

Figure 14: Damping off in nursery

Causal organisms

Various fungus like Rhizoctonia solani, Fusarium sp, Pthium sp, Phytopthora sp and so on.

Control Measures

- In cardamom nurseries special care should be exercised to get ride from this disease.
- Blitox 50 @ 2ml/ litre of water spray can save the seedlings
- Drain the excess water after irrigation. Rough out the diseased seedlings.

Make regular visits and inspection in the nursery to assess the damage.

References

Hazarika, Budhindra Nath 1* • Jaime A. Teixeira da Silva2 • Akshay Talukdar3 (2015) Effective Acclimatization of in Vitro Cultured Plants: Methods, Physiology and Genetics http://irrecenvhort.ifas.ufl.edu/plant-prop-glossary/09-tissue-culture/01-types/04-tctypesmicropropagation.html

http://vikaspedia.in/agriculture/crop-production/integrated-pest-managment/ipm-for-spice-crops/ipmstrategies-for-large-cardamom/large-cardamom-insect-and-pests-management(Accessed on 7th March, 2019)

ICIMOD (2017). Climate Resilient Practices for Sustainability of Large Cardamom Production Systems in Nepal

ICIMOD. (2016). Enhancing the Large Cardamom Production *http://www.icimod.org/?q=21571*

MoAC. (2008). Final Report: Product Chain Study Cardamom, Biratnagar. Ministry of Agriculture and Cooperatives Nepal

MoAD (2015/016). Statistical Information on Nepalese Agriculture, Government of Nepal Ministry of Agricultural Development. Monitoring, Evaluation and Statistics Division Agri Statistics Section Singha Durbar, Kathmandu Nepal.

MoAD (2017).Statistical Information on Nepalese Agriculture, Government of Nepal Ministry of Agricultural Development. Monitoring, Evaluation and Statistics Division Agri Statistics Section Singha Durbar, Kathmandu Nepal.

NSCDP. (2009). Annual Report of National Spice Crop Development Programme. National Spice Crop Development Programme, Government of Nepal, Ministry of Agriculture and Cooperative, Khumaltar, Kathmandu.

NSCDP. (2012). Annual Report of National Spice Crop Development Programme. National Spice Crop Development Programme, Government of Nepal, Ministry of Agriculture and Cooperative, Khumaltar, Kathmandu.

NSCDP. (2017). Annual Report of National Spice Crop Development Programme. National Spice Crop Development Programme, Government of Nepal, Ministry of Agriculture and Cooperative, Khumaltar, Kathmandu.

Poudel, Krishna *, 1 Prasai, Hari Kumar, ShresthaJiban (2018) *Micro propagation and Acclimatization of Large Cardamom (AmomumsubulatumRoxb.)* 1Agricultural Research Station, Pakhribas, Dhankuta, Nepal; 2National Commercial Agriculture Research Program, Pakhribas, Dhankuta, Nepal

PradhanSushen 1*, Pradhan, Smrita, Basistha,Bharat C and Subba, K B (2014) IN VITRO MICROPROPAGATION OF AMOMUM SUBULATUM (ZINGIBERACEAE), A MAJOR TRADITIONAL CASH CROP OF SIKKIM HIMALAYA

Rabgyal, Jimba 2018 Bhutan Large cardamom production manual 2018,

Tisdall, Laurence, 1989, ACCLIMATIZATION OF MICROPROPAGATED 'SILV AN'

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