# HANDBOOK OF SEED AND PLANTING MATERIAL TESTING MANUALS

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# PART 1 :
# FRUIT CROPS

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR SUBTROPICAL FRUIT CROPS

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# 1. INTRODUCTION

Healthy and good quality plant material is the foundation of successful fruit industry in the country. In view of increasing costs of labour and inputs, farming has become less remunerative. Today, farmer is in search of new alternative, especially when several incentives under Horticulture Mission and export promotion are provided by the Government. Fruit plants yield much higher than ordinary field crops and are certainly far more remunerative. It is a healthy sign that farmers are now keen to plant more areas under orchards

It is now well established that there is a need to increase area under fruit crops, in order to step up production of fruits. In India, more than 4409 fruit nurseries including 1575 under government sector 2834 under private sector, are functioning, which have an annual target of producing 1387 million fruit plants. In view of growing importance of fruit crops, the demand for quality planting material has increased manifold throughout the country in the recent past. However, the greatest bottleneck in the expansion of area under fruits is the non-availability of genuine and quality planting material in adequate quantity from reliable government nurseries. More often than not, the farmers have to get the fruit plants from unreliable sources and this practice is causing great harm to the fruit industry of the country. Some of the fruit growers, with adequate means and resources, can easily establish their own fruit nurseries with certification from a recognized government agency.

Setting up of a fruit nursery is a long term venture and needs lot of planning and expertise. Mistakes committed initially on any aspect like selection of soil, raising of right kind of cultivars/varieties, plant protection measure, etc., reduce the financial returns greatly form the investment, besides wastage of time and energy. So, careful planning is needed before setting up a nursery. The plan should show allocation of plots/area to different components of the nursery such as mother plants of different fruits/cultivars, rootstocks, roads/paths, water channels, drainage system, buildings/other

structures, etc. Provision of certain basic pre-requisites is a must for raising a fruit nursery on modern lines.

The vegetative propagation of fruit crops makes them vulnerable to transmission of several diseases and pests through the plant material. Thus, importance of testing of material in the process of its preparation at various stages needs due attentions. The huge quantity of planting material required to meet the demands of farmers/orchardists in the coming years needs coordinated approach from different ICAR institute, State Agricultural Universities and State Horticulture Departments. The targets of the enhancing fruit production in the coming years will be achieved only through production and distribution of healthy, genuine and high quality planting material of commercial/improved varieties of fruit crops in sufficient quantities. The maintenance of purity is easy in vegetatively propagated fruit crops as compared to seed propagated ones, still it requires a close monitoring at different stages in the nursery to avoid mixing with other varieties. Similarly, adequate measures are taken in the preparation of plant material to produce disease and pest free plant material.

This manual is an effort to compile the seed and planting material standards to be used for production of quality planting material of fruit crops bu different nurseries.

## 2. PROGENY TREES/MOTHER PLANTS

The bud sticks/graft wood should always be taken from healthy and true to type progeny trees of commercial/new varieties, which are free from viruses, disease and pest occurrence. A nurseryman should have progeny trees of all the promising cultivars of fruits that can be grown in that particular area.

### 2.1 Criteria for selection of mother plants

- Mother plants of the variety should be genetically true to type.
- The plants should be healthy and free from any diseases, pest infestations and physiological disorder.
- The plants should have known pedigree records regarding bearing potential, fruit quality and problems, if any.
- The plants should be precocious and prolific bearer.

### 2.2 Criteria for selection of rootstocks

i) Dwarfing /semi-dwarfing in nature

ii) Compatibility with the known commercial variety

iii) Resistance/tolerance to biotic (diseases and pests) and abiotic stresses.

iv) Rootstock should have well developed root system.

v) It should be easy to propagate rootstock by vegetative means

# 3. Protocols for propagation of different fruit crops

## 3.1 Mango (*Mangifera indica* L.)

### 3.1.1 Commercial cultivars

**North India :** Dashehari, Langra, S.B. Chausa, Lucknow Safeda, Ratol, Gaurjeet, Bombay Green, Khasul Khas

**South India :** Neelum, Banglora, Mulgoa, Suvaranarekha, Banganpalli, Rumani, Raspuri, Badami

**East India :** Malda, Fazli, Himsagar, Kishenbhog, Gulabkhas, Jardalu

**West India :** Alphonso, Pairi, Malkurad, Kesar, Rajapuri, Jamadar

**Improved varieties**

: Amrapali, Mallika, Ambika, Arka AnmolArka Aruna, Arka Neelkiran, Arka Puneet, Alfazli, AU Rumani, Ratna, Sindhu

### 3.1.2 Raising of rootstocks

Mango seedlings grown from stones of seedling trees are used as rootstocks. Stones should be collected from vigorous, disease free and high yielding trees of seedling mangoes during July-August. Mango seeds are recalcitrant and lose viability very soon on desiccation. If the mango stones are not sown within a few days of their removal from the fruit, they can be stored under moist condition in shade, covering with moist soil, sand or sawdust, etc. Before sowing stones should be immersed in water and floating stones should be discarded as they are not considered viable

Stones are sown during in June to August, depending upon the ripening season of the mango, in beds mixed with well decomposed farm yard manure at the rate of 8-10 tonnes per hectare. When the seedlings attain the age of 2-3 months, they should be transplanted in well prepared beds or polybags. After transplanting, proper care should be exercised in irrigating the young transplanted seedlings. Attack of leaf cutting insects is common during rainy season, which may affect the growth of the seedlings adversely. Care should be taken well in advance for their control before damage. In frost prone areas, the

rootstocks should also be protected from frost by thatching. Plant is allowed to grow as a single stem for six to eight months. When the plantt attains pencil thickness or a little more, it is ready for grafting.

**3.1.3 Methods of propagation** : Nurserymen in many of the mango growing areas still use inarching , traditional method of propagation. During past few decades, experimental results have shown that veneer grafting technique can be used with high success rate in Madhya Pradesh, Andhra Pradesh, Uttar Pradesh and Bihar. Stone (epicotyl) grafting is suitable for Konkan region of Maharashtra and Coastal regions. Now-a-days softwood grafting is being used commercially for mango propagation in several parts of south India. Veneer grafting and soft wood grafting techniques can be used for large scale multiplication of mango in north India. With the use of polyhouse and nethouse structures, period of propagation can be extended easily under north Indian conditions.

**3.1.4 Softwood grafting**

The technique of softwood grafting is similar to that of cleft or wedge grafting. In this case, grafting is done on 3 month to 8 month old rootstocks. In south India, the rootstocks attain graftable thickness within 3-6 months due mild winter. In the past , this technique has been in use *in situ* orchard establishment under adverse soil and climatic conditions as the grafting operation is performed using cleft/wedge method on the newly grown top portion of the plant one year after the rootstock establishment in the field . The scion shoots of the thickness equal to that of rootstocks are defoliated 7-10 days prior to grafting. The graft should be secured firmly using 1.5 cm wide, 200-gauge polyethylene strip. July and August months with high humidity and moderate temperature are the best for the success of softwood grafting.

3.1.5 **Veneer grafting** :

This method of propagation holds promise for mass scale commercial propagation. The method is simple and can be adopted with success. Eight month to one year old seedling rootstocks are suitable for this method. For conducting this grafting operation, a downward and inward 3-4 cm long cut is made in the

smooth area of the stock at a height of about 20 cm. At the base of cut, a small shorter cut is given to intersect the first so as to remove the piece of wood and bark. The scion stick is given a long slanting cut on one side and a small short cut on the other so as to match the cuts of the stock. The scion is inserted in the stock so that the cambium layers come on the longer side. The graft union is then tied with polythene strip as recommended for inarching. After the scion takes and remains green for more than 10 days, the rootstock should be clipped in stages. The scion wood to be used for veneer grafting requires similar preparation . The desired shoots should be defoliated at least one week prior to grafting so that the dormant buds in the leaf axils become swollen.

### 3.1.6 Stone or epicotyl grafting

Mango is generally propagated by inarching and veneer grafting in north India. These methods are time consuming. Stone/ epicotyl grafting is a technique of faster multiplication of mango. This method is simple, economic and fast. Fresh mango stones are sown in the nursery beds. After germination, 10-15 day old seedlings with tender stems and coppery leaves are lifted with along with stones. The roots and stones are dipped into 0.1 per cent Carbendazim solution for 5 minutes after washing the soil. The seedling stems are headed back leaving 6-8 cm long stem. A 3-4.5 cm longitudinal cut is made into the middle portion of the cut stem. A wedge shaped cut starting on both sides is made on the lower part of scion stick. The scion stick should be 4-5 months old and 10-15 cm long containing plumpy terminal buds. The scion stick is then inserted in the cleft of the seedlings and tied with polythene strip. The grafts are then planted in polyethylene bags containing potting mixture. The bags are then kept in the shade protecting from heavy rain. When the scion sprouts and the leaves become green, the grafted plants should be planted in nursery beds. July is the most suitable month for stone grafting.

3.1.7 **Care of nursery plants** : Mango plants at nursery stage are likely to be damaged by frost under north Indian conditions. So, the nursery beds should be covered covered with thatches made of sarkanda, etc. The beds should be

irrigated shenever there is danger of frost. During summer, the irrigation should be given at 4-5 day intervals depending upon the soil condition. A light application of Calcium Ammonium Nitrate or Ammonium sulphate is also recommended to encourage the growth of plants. The beds/polybags should be kept free from weeds by regular weeding/hoeing.

3.1.8 **Plant Protection Measures** :

**3.1.8.1 Insect pests :**

**Mango hopper (*Idioscopus spp.* and *Amritodus atkinsoni*)** : It is active in February –March. The nymphs and adults such the juice from tender leaves. For control, 2-3 sprays of 0.04 % monocrotophos is recommended.

3.1.8.2 **Diseases**

**Mango malformation (Bunchy top)**: On seedlings, over three months old, swellings appears in the axils of leaves. The affected plants should immediately be uprooted and destroyed. Such seedlings should not be used for rootstocks.

3.1.7 Standards for veneer grafting in mango

| S1. No. | Characters | Standards |
|---|---|---|
| 1. | Method of propagation | Veneer grafting |
| 2. | Type of rootstock | Straight & vigorous growth |
| 3. | Raising rootstock | In polyethylene bag |
| 4. | Size of polyethylene bag | 20 x 10 cm / 10 x 25 cm |
| 5. | Age of rootstock | One year old |
| 6. | Diameter of rootstock | 0.5 - 0.7 cm |
| 7. | Age of scion shoots | 3 - 4 months old |
| 8. | Diameter of scion | 0.5 - 0.7 cm |
| 9. | Length of scion stick | 15 - 18 cm |
| 10. | Plant height | 60 - 70 cm |
| 11. | Stem girth | 2.5 - 3.5 cm |
| 12. | Root type / Architecture | • Tap root<br>• Root should not be overgrown<br>• Coiling of root should not be there |
| 13. | Uniformity of grafting umon | Smooth, and union of graft 15 - 20 em above ground level/upper surface of polyethylene bag |
| 14. | Foliage | Healthy and green green |
| 15. | Disease / Pest incidence | No attack of leaf eating beetle<br>2. No die back symptom<br>No nutrient deficiency symptom |
| 16. | Precautions | • Regular watering for long transportation<br>• In the absence of polyethylene bag, the earth ball should not develop cracks<br>• Ball of earth should be properly<br>• covered with grasses |

3.1.8 Standards of soft wood grafting in mango

| S1. No. | Characters | Standards |
|---|---|---|
| 1. | Method of propagation | Wedge grafting |
| 2. | Type of rootstock | Straight & vigorous growth |
| 3. | Raising rootstock | In polyethylene bag |
| 4. | Size of polyethylene bag | 20 x 10 cm / 10 x 25 cm |
| 5. | Age of rootstock | 7 to 9 months |
| 6. | Diameter of rootstock | 0.5 - 0.9 cm |
| 7. | Age of scion shoots | 3 - 4 months old |
| 8. | Diameter of scion | 0.5 - 0.9 cm |
| 9. | Length of scion sticks | 15 - 18 cm |
| 10. | Plant height | 60 - 65 cm |
| 11. | Stem girth | 2.5- 3.0 cm |
| 12. | Union height (grafting height) | Union of graft 18 - 20 cm above ground level |
| 13. | Root type/ Architecture | Tap root |
| 14. | Foliage | Healthy and Green |
| 15. | Disease/Pest incidence | No attack of leaf eating beetle<br>No die back symptom<br>No nutrient deficiency |
| 16. | Precautions | When grafts are set out in the nursery, always handle them by holding the rootstock. Do not bump the scion, because this may disturb the callus tissue, causing the graft to die |

## 3.2 Guava (*Psidium guajava* L.)

3.2.1 Commercial cultivars : Allahabad Safeda, Sardar (L-49)

Improved cultivars : Lalit, Shweta, Arka Mridula, Arka Amulya, Dharidar, Kohir Safeda, Pant Prabhat, Safed Jam

### 3.2.2 Raising of rootstocks

Raising rootstocks in polyethylene bags is recommended as this give better establishment of plants in the field on account of undisturbed tap root system. Moreover, nursery raising in polyethylene bags saves labour in weeding, watering, shifting and lifting of plants. The chief advantage of using polyethylene bags is that, the seedlings can be raised almost round the year under controlled conditions. Guava seeds have a hard coating over the endocarp as a result of which usually long time is required for germination. Fresh seeds should be extracted from dpe fruits and washed thoroughly to remove the pulpy material clinging to the seeds. It should be treated with fungicide (copper oxychloride) to prevent damping-off of seedling before sowing in the polyethylene bag. If the damping-off occurs as the seedlings emerge, both the seedlings and the media should be treated with a fungicide. A group of fungi is responsible for damping-off of the seedlings. For controlling damping off, treatment with 0.3% copper oxychloride has been found very effective. Seeds of guava are sown in polyethylene bags (20x10 cm or 18x27cm) at any time (JanuaryDecember). Polyethylene bags are filled with soil, sand and farmyard manures in 3:1:1 ratio. All the polyethylene bags are covered with 100 micron (400 gauge) white polyethylene sheet soon after sowing of seed. During winter months, the polyethylene mulch conserves heat and create conducive environment (micro-climate) for rapid germination and early establishment of seedlings. Seed covered with polyethylene sheet gives as high as 97 per cent success within three weeks.

### 3.2.3 Patch Budding

Seedlings of about one-year- age, pencil thick, uniform and active in growth are selected. This method is most satisfactory when vigorously growing plants with 1.25-2.5 cm in stem diameter, are used as stock. The trees from which buds are

taken should be highly vegetative with lush succulent growth to permit easy separation of buds from the stem. It is better to take swollen and unsprouted dormant buds from leaf axil of mature twigs of the scion variety. A patch, approximately 1 cm (0.5 inch) to 1.5 cm (0.75 inch) with a bud seems to be taken for better success. Similarly, 1-1.5 cm long patch is removed from the rootstock and bud is fitted into the remaining portion on the stock seedling. Bud should be fitted at a height of nearly 15 em above the ground level. Polyethylene strip is used for keeping the buds close to the stock. When the bark adheres tightly to the wood, budding is usually successful. After about 2-3 weeks of budding the polyethylene strip can be opened to examine the success. In successful cases, about one-third shoot of the rootstock can be removed for forcing the growth of buds. The remaining two-thirds can be removed after three weeks of the first cutting, leaving about 2-3 em above the bud. The best time for budding is from May to August in different parts of the country.

### 3.2.4 Stooling

Stooling is the easiest and cheapest method of guava propagation. This method can be used for quick multiplication of desired varieties and also rootstocks. In this method, self-rooted plants (cuttings and layers) are planted 0.5 m apart in the stooling bed. These are allowed to grow for about three years. Then these are cut down at the ground level in March. New shoots emerge on the beheaded stumps. A 30-cm wide ring of bark is removed from the base of each shoot rubbing the cambium of the exposed portion in May. All the shoots are mounted with the soil to a height of 30 em. The soil is covered with mulch to conserve the moisture. After a period of two months of the onset of monsoon, the shoots are detached from the mother plant at ringed portion and planted in the nursery. The shoots are headed back to maintain the root and shoot balance before planting in the nursery by following the technique of ringing and mounding of the shoots, second time stooling is done on the same mother shoot in the first week of September. The rooted shoot layers are detached in the first week of November. Thus, stooling is done twice on the same mother stool in a year. The stooling of a mother stool can be done for many years. With the advancement of its age, the number of stool

layers also increases every year. The growth and development of a stool layers are better than seedlings. The application of rooting hormone is not required.

### 3.2.5 Airlayering

Air layering is one of the most important commercial method in practice for guava propagation. Rainy season (preferably July-August) is the most suitable period for air layering. In this method, limbs of about 1.2cm or more in diameter are girdled by removing a strip of bark with a width of about 2cm. The girdled area is bound with a ball of moistened sphagnum moss of about 7 em in diameter and 10-13cm long, which is then wrapped with polyethylene film and tied loosely over the wrap to prevent bird damage and also to prevent the moistened moss from overheating. Roots usually start developing in three to five weeks. When they grow through the ball of moss, the stem may be detached from the mother plant below the girdled area. The polyethylene film is removed and the new plant is potted in manured soil in pot/polyethylene bags and kept in the shade until new leaves appear. When the new growth is about 15-20cm long, the plant is hardened in full sunlight before transplanting in the field.

### 3.2.6 Soft-wood /Wedge grafting

Soft-wood /Wedge grafting, a technique for rapid multiplication has been perfected at Central Institute for Subtropical Horticulture (CISH), Lucknow. This technique has a tremendous potential for multiplying guava plants rapidly throughout the year both in greenhouse and under open conditions. Presently, the institute is producing quality materials of guava through wedge grafting technique round the year in greenhouse as well as in open conditions.

The technique involves growing of seedlings in polyethylene bags, grafting, capping and hardening of grafts. Seedlings are raised for rootstocks in the nursery for approximately 6 to 8 months. When the stem diameter of seedling is of pencil thickness (0.5-1.0 cm) they are chosen for wedge grafting.

In this technique, proper selection and preparation of scion sticks is very important for obtaining higher success. Shoot with growing apical portion

(terminal growth) which is 3 to 4 months old is ideal for this technique. The scion shoot of pencil thickness, with 3 to 4 healthy buds of 15-18 cm long is used for grafting. Selected scion shoots are defoliated on the mother plant, about 5-7 days prior to detaching. At the same time, the apical growing portion of selected shoot is also beheaded. This helps in forcing the dormant buds to swell. In this way, the buds on the scion are ready to start sprouting at the time of grafting. This treatment is essential for high success of grafts.

After selection of the scion, rootstock (seedling) is headed back by retaining 15-18 cm long stem above the soil level in the polyethylene bag. The beheaded rootstock is split to about 4.0 - 4.5 cm deep through the centre of the stem with grafting knife. A wedge shaped cut, slanting from both the sides (4.0 - 4.5 cm long) is made on the lower side of the scion shoot. The scion stick is then inserted into the split of the stock and pressed properly so that cambium tissues of rootstock and scion stick should come in contact with each other. Care must be exercised to match the cambium layer of the stock and scion along with full length of each component. The union is then tied with the help of 150 gauge polyethylene strip, 2 cm in width and 25 - 30 cm in length. Immediately after grafting, the graft is covered by 2.5 x 18.0 cm long white polyethylene cap which is tied with rubber band at the lower end. The scion starts sprouting after 9 to 12 days which is visible from out side. The cap is removed after 25 days in the evening hours. The grafts are transferred to net house for hardening.

### 3.2.7 Standards of soft wood/wedge grafting in guava

| Sl. No. | Characters | Standards |
|---|---|---|
| 1. | Method of propagation | Soft wood/Wedge grafting |
| 2. | Type of rootstock | Straight & active growth stage |
| 3. | Raising rootstock | In polyethylene bag |
| 4. | Size of polyethylene bag | 20 x 10 cm / 10 x 25 cm |
| 5. | Age of rootstock | 6 to 8 months |
| 6. | Diameter of rootstock | 0.5 – 1.0 cm |
| 7. | Age of scion shoots | 3 - 4 months old |
| 8. | Diameter of scion | 0.5 – 1.0 cm |
| 9. | Length of scion sticks | 12 - 15 cm |
| 10. | No of buds on the scion stick | 3-4 buds |
| 11. | Union height (grafting height) | 15-20 cm above the soil |
| 12. | Root type/ Architecture | Well developed root system without coiling |
| 13. | Plant height | 45-60 cm |
| 14. | Stem girth | 1.5-2.5cm |
| 15. | Foliage | Healthy and green foliage having 3 to 4 branches |
| 16. | Disease/Pest incidence | • No attack of leaf eating beetle<br>• No die back symptom<br>• No nutrient deficiency |
| 17. | Precautions | • When grafts are set out in the nursery, always handle them by holding the rootstock.<br>• Do not bump the scion, because this may disturb the callus tissue, causing the graft to die |

3.2.8 Standards of patch budding in Guava

| S1. No. | Characters | Standards |
|---|---|---|
| 1. | Method of propagation | Patch budding |
| 2. | Type of rootstock | Straight & active growth stage |
| 3. | Raising rootstock | In polyethylene bag |
| 4. | Size of polyethylene bag | 20 x 10 cm / 10 x 25 cm |
| 5. | Age of rootstock | 10 to 12 month old |
| 6. | Diameter of rootstock | 1.25 – 2.50 cm |
| 7. | Age of scion shoots | 4 - 5 months old |
| 8. | Diameter of scion | 1.25 – 2.50 cm |
| 9. | Size of patch | 1 X 1.5 cm |
| 11. | Budding height | 15-20 cm above the soil/ground |
| 12. | Stem girth | 3.5-4.5 cm |
| 13. | Bud union | Smooth |
| 14. | Plant height | 40-60 cm |
| 15. | Root type/ Architecture | Well developed root system without coiling |
| 16. | Foliage | Healthy and green foliage having 3 to 4 branches |
| 17. | Disease/Pest incidence | • No attack of leaf eating beetle<br>• No die back symptom<br>• No nutrient deficiency |
| 18. | Precautions | • When grafts are set out in the nursery, always handle them by holding the rootstock.<br>• Do not bump the scion, because this may disturb the callus tissue, causing the graft to die |

### 3. 3 Aonla (*Emblica officinalis* Gaertn)

**3.3.1 Commercial Cultivars :** Chakiya, Kanchan, Krishna, Narendra Aonla-6, Narendra Aonla-7, Narendra Aonla-10

### 3.3.2 Raising of rootstocks

Aonla is commercially propagated by budding/ grafting on seedling rootstock. Fruits are collected from local seedling (desi) aonla trees and used for rootstock raising. Mature fruits should be collected during January/February. Fruits are dried in open and seeds are extracted by applying light pressure. One kg seed can be obtained from one quintal of desi aonla fruits. Raising of seedling is essential for rootstock. The timing for sowing of seed has been standardized. Sowing of seed on raised bed (after soaking in water for 12 hours) or in poly bag during March/ April facilitates quick germination. Germination of seeds of aonla is better during March-April and July-September.

### 3.3.3 Patch Budding

Six months to one-year old seedlings are used as rootstock for budding. The scion shoots should be selected from the mother plants, which are prolific bearers and free from disease and pest incidence. Patch/ modified ring budding during mid of May to September gives 60 to 90 per cent success under north Indian conditions. However, in south India, aonla propagation is being done almost 8-10 months in a year with the aid of greenhouse and nethouse facilities. Besides budding, veneer and soft wood grafting are also successfully attempted with about 70 per cent success. However, considering the efficiency, budding appears to be an ideal method for aonla propagation.

Propagation of aonla in poly bags/poly tubes, or *in situ* orchard establishment (particularly in the drier areas) have been standardized and needs popularization. Aonla scion shoots can safely be stored/ transported in sphagnum moss / moist newspaper for 5-7 days with ample success.

### 3.3.4 Soft wood/Wedge grafting

When the seedling attains pencil thickness, it is ready for grafting. The top of the

rootstock is cut off at the height of 15-18 cm from the surface of poly bag or ground. Splitting the beheaded rootstock vertically down the center, to a point 4 to 5 cm below the cut surface. Scion stick is collected from desired variety. The shoot with 6 to 8 healthy buds, 12 to 18 cm long pencil thick is cut from the selected mother plant. Scion stick should be cut from both sides into a tapering wedge approximately 4 to 5 cm long. The tapered end is inserted into the split stem of the rootstock.

The rootstock and scion are wrapped tightly with 2 cm wide and 25 to 30 cm in length polyethylene strip. Immediately after grafting, the scion is covered with poly cap. Within 12 to 15 days of grafting scion shoots sprout, which is visible from outside. The polycaps are carefully removed after 21 days and these are kept for hardening. Early removal of poly caps results in high mortality. Winter months suitable for wedge grafting in the field conditions, while round the year can be grafted in greenhouse. Field transferable grafts become ready within 6-8 months of seed sowing. This method ensures 100 per cent establishment and survival of transplants in the field on account of undisturbed root system. This method also ensures the authenticity of the planting material which has been grafted. Budding and wedge method of propagation is the appropriate technique for mass multiplication of aonla plants.

### 3.3.5 Standards of patch budding in aonla

| S1. No. | Characters | Standards |
|---|---|---|
| 1. | Method of propagation | Patch budding |
| 2. | Type of rootstock | Straight & active growth stage |
| 3. | Raising rootstock | In polyethylene bag |
| 4. | Size of polyethylene bag | 20 x 10 cm / 10 x 25 cm |
| 5. | Age of rootstock | 5 to 7 month old |
| 6. | Diameter of rootstock | 0.80 – 1.25 cm |
| 7. | Age of scion shoots | 3 - 5 months old |
| 8. | Diameter of scion | 0.80 – 1.25 cm |
| 9. | Size of patch | 1 X 1.5 cm |
| 11. | Budding height | 15-20 cm above the soil/ground |
| 12. | Stem girth | 3.5-4.5 cm |
| 13. | Bud union | Smooth |
| 14. | Plant height | 40-60 cm |
| 15. | Root type/ Architecture | Well developed root system without coiling |
| 16. | Foliage | Healthy and green foliage having 3 to 4 branches |
| 17. | Disease/Pest incidence | • No attack of leaf eating beetle<br>• No die back symptom<br>• No nutrient deficiency |
| 18. | Precautions | • When grafts are set out in the nursery, always handle them by holding the rootstock.<br>• Do not bump the scion, because this may disturb the callus tissue, causing the graft to die |

3.3.6 Standards of soft wood/wedge grafting in aonla

| S1. No. | Characters | Standards |
|---|---|---|
| 1. | Method of propagation | Soft wood/Wedge grafting |
| 2. | Type of rootstock | Straight & active growth stage |
| 3. | Raising rootstock | In polyethylene bag |
| 4. | Size of polyethylene bag | 20 x 10 cm / 10 x 25 cm |
| 5. | Age of rootstock | 6 to 9 months |
| 6. | Diameter of rootstock | 0.5 – 1.0 cm |
| 7. | Age of scion shoots | 3 - 4 months old |
| 8. | Diameter of scion | 0.5 – 1.0 cm |
| 9. | Length of scion sticks | 12 - 15 cm |
| 10. | No of buds on the scion stick | 6-8 buds |
| 11. | Union height (grafting height) | 15-20 cm above the soil |
| 12. | Root type/ Architecture | Well developed root system without coiling |
| 13. | Plant height | 45-60 cm |
| 14. | Stem girth | 2.0-2.5cm |
| 15. | Foliage | Healthy and green foliage having 3 to 4 branches |
| 16. | Disease/Pest incidence | • No attack of leaf eating beetle<br>• No die back symptom<br>• No nutrient deficiency |
| 17. | Precautions | • When grafts are set out in the nursery, always handle them by holding the rootstock.<br>• Do not bump the scion, because this may disturb the callus tissue, causing the graft to die |

## 3.4 Bael (*Aegle marmelos* Corr.)

| **Name of the crop** | Bael |
|---|---|
| **Botanical Name** | *Aegle marmelos* Corr |
| **Information regarding mother plants** | True to type, precocious, high yielding mother plants of the variety |
| **Commercial cultivars** | Narendra Bael-5, NB-7, NB-9, CISH B-1, CISH B-2, Pant Aparna, Pant Sujata, Pant Shivani Pant Urvashi, |
| **Method of Propagation** | Vegetative propagation |
| **Method of Vegetative propagation** | 1. Patch budding and soft wood grafting |

### 3.4.2 Standards for Patch budding in bael

| | |
|---|---|
| Type of rootstock | Straight & active growth stage |
| Raising rootstock | In polyethylene bag |
| Size of polyethylene bag | 25 X 10 cm |
| Age of rootstock | 8 to 12 month old |
| Diameter of rootstock | 0.70 – 1.20 cm |
| Age of scion shoots | 3 – 6 month old |
| Diameter of scion | 0.70 – 1.20 cm |
| Size of patch | 1 X 1.5 cm |
| Budding height | 15-20 cm above the soil/ground |
| Bud union | Smooth |
| Plant height | 40-60 cm |
| Root type/ Architecture | Well developed root system without coiling |
| Foliage | Healthy and green foliage having 3 to 4 branches |
| Disease/Pest incidence | No attack of leaf eating caterpillars |
| Precautions | • Shifting polybags in nursery is required at intervals to discourage tap root.<br>• Grafts are to be handled carefully to avoid damage to the union or scion portion. |

3.4.3 Standards for soft wood grafting in bael

| Method of propagation | Soft wood/Wedge grafting |
|---|---|
| Type of rootstock | Straight & active growth stage |
| Raising rootstock | In polyethylene bag |
| Size of polyethylene bag | 25 X 10cm |
| Age of rootstock | 6 to 8 months |
| Diameter of rootstock | 0.7 – 1.0 cm |
| Age of scion shoots | 3 - 6 months old |
| Diameter of scion | 0.7 – 1.0 cm |
| Length of scion sticks | 12 - 15 cm |
| No of buds on the scion stick | 5-8 buds |
| Grafting height | 15-20 cm above the soil |
| Root type/ Architecture | Well developed root system without coiling |
| Plant height | 45-60 cm |
| Foliage | Healthy and green foliage with vigorous growth |
| Disease/Pest incidence | No attack of leaf eating caterpillars |

## 3.5 Papaya (*Carica papaya* L.)

| | |
|---|---|
| **Name of the crop** | Papaya |
| **Botanical Name** | *Carica papaya* L. |
| **Information regarding mother plants** | True to type plants producing high yields and quality fruits |
| **Commercial cultivars** | Pusa Delicious, Pusa Majesty, Pusa Nanha, Pusa Dwarf, Pusa Giant, Coorg Honey Dew, Co. 1, Co. 2, Co. 3, Co. 4, Co. 5, Co. 6, Co. 7, Sunrise Solo, Washington, Surya |
| **Method of Propagation** | Papaya is propagated by seed. Seed should be fresh as its viability is lost in about 45 days. |
| **Method of seed production** | Sib Mating |
| **Method of Sib Mating** | • To maintain the purity of papaya seeds, sib-mating is necessary ie., mating of sister and brother plants from the same parent.<br>• In this method, seeds from the fruits of a tree which show the typical parental qualities of the tree, i.e. leaves and fruits are first selected.<br>• The seeds of these fruits are sown and the seedlings transplanted in the main field and when these plants put forth flowers, progenies conforming to the characters which formed the basis for the original selection are marked among the female as well as male plants.<br>• Pollens from the male plant is taken and put on the stigma of female flowers one day ahead of opening of the flowers.<br>• The female flowers are then covered with butter paper bags to prevent their contamination with other flowers. It is marked with a tag.<br>• The fruit sets in about five to seven days when the bag is removed. When these sib-mated<br>• fruits mature, they are harvested and the seeds are extracted from raising second generation of plants.<br>• This process is continued from generation to generation while the entire |

| | |
|---|---|
| | population raised from the in-breds show uniformity of characters for which selections were originally made. |
| **Seed rate** | 250-300 g per hectare |
| **Soil treatment** | Formaldehyde treatment followed by covering with polythene sheet for 4-5 days and leaving it further exposed for 3-4 days |
| **Seed treatment** | Seed treated with captan @ 3g/kg seed |
| **Seed bed size** | 3 m (length)X 1m (Width) X 10-15 cm (Height) |
| **Polythene size** | 20 X 15 cm or 25 X 10 cm |
| **Polythene mixture** | Soil : sand : FYM in 1 : 1 : 1 ratio |
| **Depth of sowing** | 1-2 cm |
| **Spacing between row to row** | 10-15cm |
| **Time of sowing** | August-September under north Indian conditions |
| **Irrigation** | Frequent light irrigations as per requirement |
| **Weeding** | From time to time as per requirement |
| **Standards of the planting material** | |
| Height of the plant | 15-30 cm |
| **Age of the plant** | One month |
| Growth of the plant | Vigorous |
| Root system of the plant | Well developed without coiling of roots |
| **Disease incidence** | |
| Name of the disease | Damping off |
| Causal organism | Fungal disease |
| Detection and diagnosis | Serious disease in nursery, causes both pre and post emergence death of seedlings in nursery |
| Control | Seed treatment with thiram or captan<br>Drenching of nursery with formaldehyde, 0.4 % thiram or captan |

**Annexure-1**

## PROPAGATION METHODS OF FRUIT CROPS-AT A GLANCE

| S. No. | Name of the fruit species | Commercial methods of propagation | Rootstocks recommended |
|---|---|---|---|
| 1. | Almond (*Prunus amygdalus*) | 'T' budding | Peach and bitter almond seedlings |
| 2. | Amra (*Spondias mangifera*) | Seed, cutting | - |
| 3. | Aonla (*Emblica officinalis*) | Patch budding, soft wood grafting | Wild seedlings |
| 4. | Apple (*Malus domestica*) | Tongue and whip grafting | Clonal rootstocks, such as M-9, M-27, MM106, Mm-104, etc |
| 5. | Apricot (*Prunus armeniaca*) | 'T' budding | Apricot and peach seedlings |
| 6. | Avocado (*Persea Americana* Mill.) | Cleft grafting, Chip and 'T' budding | |
| 7. | Barhal (*Artocarpus lakoocha*) | Seed, Airlayering | - |
| 8. | Bael (*Aegle marmelos*) | Patch budding, soft wood grafting | Seedlings |
| 9. | Banana (*Musa spp*) | Sword suckers, tissue culture | - |
| 10. | Carambola (*Averrhoa carambola*) | Soft wood grafting | Seedlings |
| 11. | Cashewnut (*Anacardium occidentale*) | Soft wood grafting | Seedling |
| 12. | Cherry (*Prunus avium*) | 'T' budding | MF 12/1, Morelo, Mazzard |
| 13. | Chestnut (*Castania sativa*) | Stooling | - |
| 14. | Chilgoza (*Pinus gerardiana*) | Seed | - |
| 15. | Chironji (*Buchanania lanzan*) | Seed | - |
| 16. | Coconut (*Cocos nucifera*) | Seed | - |
| 17. | Custard apple (*Annona squamosa*) | Veneer and soft wood grafting | Seedlings |
| 18. | Date palm (*Phoenix sylvestris*) | Offshoots | - |
| 19. | Durian (*Durio zibethinus* Merr.) | Budding, Wedge grafting | Seedlings |
| 20. | Grape (*Vitis vinifera*) | Hard wood cutting | - |
| 21. | Guava (*Psidium guajava*) | Air layering, patch budding, soft wood/wedge grafting | Seedlings |
| 22. | Gulabjamun (*Syzygium jambos*) | Air layering | - |
| 23. | Hazelnut (*Coryllus avellana*) | Tongue grafting | Wild seedling |
| 24. | Jackfruit (*Artocarpus heterophyllus*) | Patch budding | seedling |
| 25. | Jamun (*Syzygium cumini*) | Patch budding, Soft wood grafting | Local seedlings |
| 26. | Kagzi lime (*Citrus aurantifolia*) | Seed | - |
| 27. | Karonda (*Carissa carandas*) | Seed | - |
| 28. | Khirni (*Manilkara hexandra*) | Seed, Veneer grafting | Seedling |
| 29. | Kiwifruit (*Actinidia deliciosa*) | Hard and Semihard wood cutting | - |
| 30. | Lasora (*Cordia myxa*) | Seed, Patch budding | Seedlings |
| 31. | Lemon (*Citrus limon*) | Air layering | - |
| 32. | Litchi (*Litchi chinensis*) | Air layering | - |

| 33. | Longan (*Dimacarpus longa* ) | Seed | - |
|---|---|---|---|
| 34. | Loquat (*Eriobotrya japonica*) | Cleft grafting , patch budding | Seedlings |
| 35. | Macadamia Nut *(Macadamia integrifolia)* | Tongue, splice, cleft, side-wedge, Root cuttings | Seedlings |
| 36. | Mahua (*Bassia latifolia*) | Seed, Veneer grafting | Seedling |
| 37. | Mandarin (*Citrus reticulate*) | Budding | Seedlings of Rough lemon and Rangpur lime |
| 38. | Mango (*Mangifera indica*) | Veneer grafting, stone grafting, soft wood grafting | Seedlings |
| 39. | Mangosteen (*Garcinia mangostana* ) | Seed, Cleft grafting | - |
| 40. | Mulberry (*Morus spp*) | Hard Wood Cutting | - |
| 41. | Papaya (*Carica papaya*) | Seed | - |
| 42. | Peach (*Prunus persica*) | T-Budding | Peach seedlings |
| 43. | Pear (*Pyrus communis*) | T-Budding, Whip grafting | Mehal, Quince-A, B and C |
| 44. | Pecannut (*Carya illinoensis* Koch) | Patch budding, cleft grafting | Pecan seedlings |
| 45. | Persimmon (*Diospyros kaki*) | Crown grafting | - |
| 46. | Phalsa (*Grewia subinaequalis* | Seed | - |
| 47. | Pine apple (*Ananas comosus*) | Suckers, slips | - |
| 48. | Pistachionut (*Pistacia vera*) | Seedling | - |
| 49. | Plum (*P. domestica)* | 'T' budding, tongue grafting | Damas-C, Pixy, Myrobalan |
| 50. | Pomegranate (*Punica granatum)* | Hard wood cutting | - |
| 51. | Rambutan (*Nephelium lappaceum* L.) | Seed, Cleft grafting, Shield or T budding | Seedlings |
| 52. | Sapota (*Manilkara achras*) | Soft wood grafting | Khirni seedlings |
| 53. | Strawberry (*Frageria chiloensis*) | Stolons | - |
| 54. | Sweet orange (*Citrus sinensis*) | Shield/'T' Budding | Seedlings of Rough lemon and Rangpur lime |
| 55. | Tamarind (*Tamarindus indica*) | Patch budding, Soft wood grafting | Seedlings |
| 56. | Walnut (*Juglans regia*) | Patch budding | Wild seedlings |
| 57. | Wood apple (*Feronia limonia*) | Soft wood grafting | Seedlings |

# MANUAL
# OF
# PLANT
# PROPAGATION

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**Introduction:**

The production of consistent quality planting material requires a quality control programme to manage key production variables in the horticultural nurseries. There are many variables in the production of nursery plants, which need to be identified and controlled; otherwise the quality of planting material would probably be erratic. However, little efforts have so far been made on a systematic basis in this direction and there is no explicit and dependable quality control programme on hand in India for planting material production. Hence, it is necessary to develop a manual for propagation for ensuring quality of planting material in the country, which is very important for enhancing productivity and production of fruit crops.

**Preliminary considerations:**

There are several key steps to initiate a quality control programme for horticultural planting material production. The first step would be to:

1. Identify the potential for planting material production and to set realistic production targets based on the resource availability. Availability of sufficient resources, particularly the genuine and certified scion / budwood and rootstock according to the scientific recommendations in vogue as well as that of skilled manpower for the propagation methods to be followed should be the major considerations for setting realistic targets, from a quality control point of view.
2. Location of targeted planting material production as selection of an ideal location can also serve to improve production quality considerably. In general, it should be located at a site with access to modern communication and transport facilities, good water source, electricity, skilled and unskilled labour availability throughout the season as well as professionally qualified and competent manpower to oversee the production and quality control. Places with a mild climate, long growing season and even distribution of rainfall is most suitable for planting material

production while areas with extremes of temperature or commonly subjected to dry winds, frequent flooding, hail, storms or are known to be frost pockets are better avoided as they adversely affect the quality of planting material. Soil should preferably be light to medium in texture with good fertility, water holding capacity and drainage, ideally with a pH range of 6.0 to 7.0. Topography should preferably be plain with gentle slope (1 to 2 %); and in very sloppy areas terracing need to be done.

3. Develop a Simple Flow Chart with Time Scale for the Production Process to meet the production target, wherein the major production steps need to be identified that will vary with the type of planting material produced. The major considerations in this regard would be propagation method and its seasonal variations for success and quality of the final produce, rootstock to be used, and specifications for the scion / bud wood including its genuineness and seasonality of availability.
4. Identify the inputs necessary for each stage of production and develop specifications and requirements for each input. These generally include containers, growing medium, fertilizer (liquid feed or granular; if granular, regular release or control release), irrigation water, plant growth regulators, pesticides, herbicides, etc. The specifications for each input should be two or three features critical to the production of quality planting material and should be quantitative (that can be measured and verified, Example: container - black polybags 300 gauge thickness, 24 cm x 18cm size with 4 to 6 holes 5 cm above the bottom; irrigation water - less than 0.5 $dsm^{-1}$ EC, less than 100 ppm $CaCO_3$ content; etc). The specifications for size and durability of containers should be based on the duration the planting material has to retained in them without becoming pot-bound and without any bending or twisting of the taproot. Containers should have provision for drainage of excess water. The widely used growing medium in fruit crop nurseries at present is a mixture of sand, FYM and red earth in equal proportions. This medium needs sterilization by treatment with heat (steam or solarization) or chemicals (such as formaldehyde) to get rid of inherent soil borne pathogens such as *Fusarium*, *Phytophthora* etc and pests such

as nematodes. Afterwards, the sterilized medium has to be inoculated with beneficial soil microorganisms such as Vesicular Arbuscular Mycorrhizae (VAM) and Phosphate Solubilizing Bacteria (PSB), supplemented with rock phosphate / super phosphate, filled into the containers and planted with the seed / seedling / cutting. Colonization of the beneficial microbes in the root zone can be verified forty-five days after the inoculation. Use of commercial sterile potting mixes can avoid sterilization of growing medium. Coco-peat, a byproduct of coir industry, has high water holding capacity and good texture, but is low in mineral nutrients. Organic materials such as leaf mold can also be used for preparing growing media, but its composition can vary widely making quality control difficult. The points to be considered while developing specifications for growing medium of nursery plants are proper drainage and water holding capacity, ability to supply requisite plant nutrients, freedom from pests and disease inoculums and proper texture to facilitate root growth. Handling and storing the media inside potting sheds helps to prevent their quality deterioration. The quality of irrigation water with respect to its pH, EC, and dissolved salts has to be defined based on the sensitivity of the plant species under consideration to these and has to be checked at frequent intervals, especially during summer.

5. Develop procedures to verify that each input meets the specification standards**.** The procedures developed will be critical to the success of quality control programmes. Ideally, the procedure to verify would be real-time, for immediate recognition that the input meets specifications. Also, the information collected should be quantitative, so as to assess variation that might occur. The verification procedures should be an integral part of the routine production procedures with an established frequency of measurement that can vary from every time the input is applied, to once a season or so. An appropriate procedure might call for periodic measurement of microbial load, soluble salts and pH of the water and media prior to use in production while container label of fertilizers and pesticides may be checked for content and active ingredient concentration while procuring and the composition of the fertilizer / pesticide solution should be verified prior to use.

6. Proper nursery records may be maintained incorporating all the above information either in the registers and / or in the computer using suitable software such as Excel or MS Access, for monitoring the quality control programme. This would be also useful in identifying probable flaws in the programme retrospectively and to rectify them subsequently. At every step, continuous and effective coordination with research organizations on the latest technology development regarding the nursery management aspects would be helpful to upgrade and perfect the quality control measures.
7. Labeling of each planting material produced properly as per the records, with necessary details such as crop, variety, rootstock used, date of production, name of the nursery etc and each batch may be certified by the competent authority for compliance with the quality control programme.

**Bio-fertilizers in Nursery Management:**

Bio-fertilizers are not alternatives for inorganic fertilizers. But they are useful in increasing yield, quality and yield of crops when they are used in combination with organic manure and inorganic fertilization in a balanced proportion. Bio-fertilizers used in horticulture nursery can be grouped under three distinct categories such as 1. **Nitrogen fixers,** 2. **Phosphate solubilisers** and 3. **Mycorrhizal fungi.**

**1. Nitrogen fixers:** The nitrogen fixing organisms associated with horticultural crops are the Rhizobium species associated with leguminous crops and the free living micro-organisms belonging to the *Azotobacter* Spp. and the *Azospirillum Spp*. which live in association with the root system of non leguminous horticultural crops.

***Azospirillum: Azospirilla*** a group of bacteria found in association with the root system of many crop plants. It is a type of symbiosis where the bacterial cells are found colonizing the root cortical cells or the intercellular spaces in the cortex. These bacteria grow better under reduced oxygen levels. They fix nitrogen from 10 to 40 kg per hectare. They are found colonizing the root system of many vegetable plants. *Azospirillum* inoculation helps the plants in better vegetative growth and also saves nitrogenous fertilizers upto 25-30%.

***Azotobacter:*** *Azotobacter* are another free living aerobic nitrogen fixing bacteria which can substitutes part of inorganic fertilizers. *Azotobacter* inoculation saves addition of

nitrogenous fertilizers by 10 to 20 %.

**2. Phosphate Solubilising Micro-organisms :** Phosphorus is a very important nutrient of plants required in large quantities. Inorganic forms of this nutrient are compounds of Ca, Fe, and Al. A large amount of phosphorus applied to various soils gets fixed and makes it unavailable to the plants. Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* and fungi belonging to the genera *Penicillium* and *Aspergillus* possess the ability to convert insoluble phosphates in soil into soluble forms by secreting organic acids. These acids lower the pH and dissolves of bound form of phosphate. Some of the hydroxy acids may chelate with Ca and Fe resulting in effective solubilisation and utilization of phosphates by crops.

**Methods of inoculation**: There are several methods of applying bacterial inoculants to the crops :

(1) Seed treatment, (2)Treating the nursery soil (3) Treating the roots of seedlings before transplanting

**Seed treatment**: *Rhizobium Azospirillum , Azotobacter* and PSM inocula which are supplied in certain carrier base like charcoal powder or lignite powder is coated to the seeds, dried under shade and directly sown in the nursery.

**Nursery treatment:** They can be also added to the nursery in rows and seeds are sown over them

**Treatment of seedlings:** Seedlings taken from the nursery may be dipped in a slurry made out of the inoculum before transplanting to the main field.

Azospirillum and Azotobacter culture can be applied near the zone where roots develop and chemical fertilizers can be applied 15 to 20 days later at 10 to 15 cm depths.

**3. Mycorrhizal fungi :**

The vesicular arbuscular mycorrhizae (VAM) are formed by the fungi belonging to the genera *Glomus, Gigaspora, Acaulospora* and *Sclerocystis.* They produce vesicles and arbuscules inside the root system. Arbuscules are highly branched fungal hyphae while vesicles are the bulbous swellings of these hyphae. These VAM fungi make more nutrients available to the host plant, improve soil texture, water holding capacity, disease resistance and help in better plant growth. Besides, mycorrhizae are also helpful

in the biological control of root pathogen .

**Field trials with VAM inoculum**: In fruit crops like mango, citrus, papaya, pomegranate and all vegetable crops and ornamental crops which are raised in nursery VAM is inoculated in the nursery itself and the VAM colonised cuttings or seedlings are then transferred to pits in the main field.

**Method of inoculation:**. VAM inoculum is added to FYM + soil + sand mixture, 1:1:1 at the rate of 50 g inoculum per 500 g of soil mixture per bag. Seeds of papaya, or vegetables or ornamental crops are planted in each bag. Within 45 days, root system of the plants will get colonised by VAM and ready for

**Testing the plants for VAM colonisation** : 45 days after inoculation the root system is tested for the colonisation of the fungus. The freshly emerged feeder roots are cut, washed in water and treated with 10% KOH and heated at $90^0$ C or at 15 lbs pressure for 5 minutes. In case of hard root system, the heating may be for more time. Then wash the roots for 10 minutes with hydrogen peroxide when the roots are pigmented masking the arbuscules.

**VAM Inoculum Production:**

**On-farm inoculum production techniques** : Large-scale inoculum production is done on-farm by following procedure.

Select a land near the field to be cultivated. After ploughing, harrowing and cultivator operations, cover the soil with polythene sheet for 30 days in bright sunlight or fumigate with 2% formaldehyde or methyl bromide to sterilise the soil. Remove the polythene sheet and apply the starter cultures of the VAM fungi at a depth of 3-4 cm from the surface .Sow the seeds of Rhodes grass or Ragi or any other crop with good root system and cover with the soil. Irrigate field regularly. When the crops attain a height of 10-15 cm viz.,12 weeks after planting cut off the shoot at the ground level. The soil along with the root system is used upto 20-cm depth as inoculum. base. Reduce the application of phosphorus fertilizers by 25-50% depending upon the crop. The plants get colonised upto 80-90% within 3-8 months. The nutrient contents like P, Zn and Cu are increased in the leaves. We can save Phosphorus by 25-50% without reduction in the yield of plants.

Vegetable crops are inoculated with VAM fungi in the nursery beds. The inoculum, consisting of spores of the fungus with the soil and the infected root bits of the host plants, is either spread uniformly in the nursery bed or placed in rows over which seeds of tomato, brinjal, chillies or capsicum are sown. The fungus colonises the seedlings within 45 days, which are transplanted to the main field. The inoculated plants of these vegetables show better growth and improved nutrient content. By VAM application addition of inorganic phosphates can be reduced by about 25-50% depending upon the soil phosphorus content.

**Nematode control with mycorrhiza**: Mycorrhizal fungi help in nematode control also. When botanicals like Neem cake are used along with mycorrhiza nematode infection can be brought down by 60%.Mycorrhizal fungi act synergistically with *Azotobacter and Azospirillum*. Legumes inoculated with these bacteria show better nodulation, nitrogen fixation and yield.. The mycorrhizal inoculum may be dibbled into the soil along with the seed. It can also be mixed with FYM and broadcasted in the main field before sowing the seeds of legume crop.

Bio-fertilizers suitable to different horticultural crops along with their method/ stage of application are given in table1 and table 2.

**Precautions to be taken while using Bio-fertilizers**:

- Seeds coated with Bio-fertilizers should not be treated with pesticides as there are possibilities of the microorganisms being killed by them.
- Preserve the Bio-fertilizers away from sunlight, heat and moisture. Store them in cool place away from any pesticides.
- Bio-fertilizers should be used before the expiry date as they get inactivated after expiry date resulting no response when applied to crops.
- Chemical fertilizers and bio-fertilizers should not be applied together as the micro-organisms in bio-fertilizer will get killed due to the plasmolysis

**Seedling Production:**

A good quality seedling production is very essential for getting higher yield and quality of produce. Vegetable seedling production is a specialized activity and farmers buy the seedlings from these nurseries. In India too, vegetable seedling production system has changed in recent years in some of the intensive vegetables growing areas and seedling production as a specialized practice is fast catching up.

**Seedling Production using Seedling Trays:**

It is already a commercial venture to produce the seedlings of tomato, capsicum, cauliflower and cabbage hybrids using seedling trays and protective structure. In the past, the farmers themselves use to produce all seedlings used for transplanting. This was all right as most of them were growing comparatively low cost open pollinated vegetable varieties. Now a days many progressive farmers are coming forward to take up quality seedling production using seedling trays and supply to the individual farmers. Even for fruit crops like papaya bigger sized trays can be used for raising the seedlings. This method is mostly adopted for raising seedlings of $F_1$ hybrids since the cost of the seed is quite high. The vegetable seedlings are produced under protective structures such as insect proof net houses, shade houses and low cost naturally ventilated greenhouses.

**i) Advantages of seedling rising through seedling trays:**

1. Growing in seedling trays with right growing media helps in proper germination.
2. It provides independent area for each seed to germinate and grow.
3. Seedling mortality or damping off rates are reduced by using properly sterilized growing media.
4. Uniform and healthy growth of all seedlings.
5. Easy in handling and economy in transportation.
6. The use of trays results in win-win situation – as the grower gains and saves a lot on expensive seeds.
7. Root development is better and root damage while transplanting is nil. Thus better transplant establishment and crop stand.
8. Uniform and early maturity.

**ii) Protected structure for seedling raising:**

The seedling trays are commonly kept under nylon net house or poly house. Net house is found to be cost effective and feasible structure to grow vegetable seedlings. Net house is commonly built using granite stone pillars. Stone pillars of 10’ x 6’’ x 4’’ are generally used. These stone pillars are spaced at 5m x 5m and grouted to a depth of 2

feet. The stone pillars all along the periphery of the net house should be tied to a peg stone using guy wire. The height of the structure should be 8 feet. On top of each stone pillar used rubber tube is tied so that sharp edges of the pillars do not damage the nylon mesh and shade net. Wire grid is provided at the top of the structure as support for the nylon mesh. Normally farmers cover the sides with 40 mesh UV stabilized nylon insect proof net and in the top 50% UV stabilized HDPE shade net is used to cover the net house. It is recommended to cover the sides and top of the net house with 40 mesh UV stabilized nylon insect proof net. During summer and hot sunny days 25 % or 35% UV stabilized HDPE shade net is spread over the nylon mesh on the top of the net house to maintain ambient temperatures suitable for crop growth. Provision should be made to pull polythene sheet over the pro-trays in the event of rainfall by way of making low tunnel structure. For preparing low tunnel structure, 3/4" HDPE pipes or bamboo stick and 400-gauge polyethylene sheet can be used. The approximate cost for building stone pillar net house will be Rs. 80 to Rs. 100 per square meter depending on the locality. Seedling rising can also be done in low cost greenhouse or wooden playhouses.

**iii) Seedling trays:**

1. Seedling trays are also called as pro-trays (propagation tray) or flats, plug trays or jiffy trays.
2. The dimensions of the trays generally are 54 cm in length and 27 cm in breadth and cavity depth of 4 cm. These trays are made of polypropylene and re-usable.
3. Life of the tray depends on the handling the seedling trays.
4. Seedling trays have been designed in such a way that a sapling gets a pre calculated growing media and the right amount of moisture as the trays have pre punched holes to each cavity for proper drainage of excess water and also right spacing.

**iv) Growing media for seedling trays:**

1. Sterilized commercial growing media is better as the incidence of seedling diseases is less or nil and it contain right amount of moisture in it.

2. The most common growing media used is coco peat, which is steam sterilized to prevent nursery diseases.
3. Coco peat is a by-product of coir industry and it has high water holding capacity. It should be well decomposed, sterilized and supplemented with major nutrient sources before using. Basically coconut fibre powder is low in nutrients and high in lignin content. Thus it need to be properly decomposed by adding major and micronutrients and microorganisms.
4. Other growing media which have given good result are cocopeat: vermi-compost, vermi-compost: sand in equal proportions.

**v) Method of seedling rising:**

1. Fill the seedling tray with appropriate growing medium such as coco peat.
2. Make a small depression for sowing (0.5 cm) by fingertip in the center of the cell. Alternatively, depression can be created by stacking about 10 trays one over other and pressing the trays together.
3. Sow one seed per cell and cover by coco peat.
4. No irrigation is required before or after sowing if coco peat having 300-400 percent moisture is used.
5. Keep about 10 trays one over the other for 3 to 6 days, depending on the crops. Cover the entire stack of tray with polyethylene sheet. This arrangement ensures the conservation of moisture in the seedling trays until germination and hence no irrigation is required till seedling emergence. Care must be taken for spreading the trays when the seedling is just emerging, otherwise seedlings will get etiolated.
6. Seeds start emerging after about 3-6 days of sowing depending upon the crops. Then the trays are kept spread over a bed covered with polyethylene sheet.
7. The germinating trays are then irrigated lightly, daily depending upon the prevailing weather conditions.
8. The trays are also drenched with fungicides as a precautionary measure against seedling mortality.
9. Seedling trays are watered daily, or as needed (not too wet or too dry) using a fine sprinkling water can with rose or with hose pipe fitted to rose.

10. Do not over irrigate the trays; if done will result in the leaching of nutrients and help in building up of diseases.
11. The media may need to be supplemented with the nutrient solution if the seedlings show deficiency symptom. Spray apply 0.3 per cent (3g/litre) of 100 percent water soluble fertilizer (19 all with trace elements) twice (12 and 20 days after sowing).
12. Protect the trays from rainfall by covering the polyethylene sheets in the form of low tunnel.
13. Harden the seedlings by with holding irrigation and reducing the shade before transplanting

**vi) Use of Nylon Net:**

It is important to have vegetable seedlings that are free of insect pests and disease problems. The earlier the plants are infected with pests or diseases, the more severe the effect on the field crop growth and yield. In this direction, growing vegetable seedling under cover using insect proof nylon net (40-50 mesh) is a good practice. Use *Casuarina* or bamboo poles or GI pipe to support the net to be used by a small farmer. UV stabilized and properly stitched nets will last for 6-8 years.

**ii) Solarization for nursery bed sterilization:**

It is a method of heating soil through sunlight by covering it with transparent polythene sheet to control soil borne diseases including nematodes. This method used for the disinfection of raised nursery bed made in soil to produce healthy seedlings of vegetable. Other additional beneficial effects include control of weeds, insect pests and release of plant nutrients resulting in increased crop growth. Solarization is a non-chemical alternative for disease, insect pest and weed control.

**Method of soil solarization:**

1. Prepare the raised bed, add organic manure and make the bed ready for sowing in every respect.

2. Bring the bed to field capacity by irrigating. Cover the nursery beds with 200 gauge transparent polyethylene film as tightly and closely to the ground as possible.
3. Leave the beds covered for 30-40 days. The soil temperature of the nursery bed thus covered can go up to 52$^{o}$C in summer months.
4. Check the sheets for the tear and if found torn out, seal with transparent tapes.
5. After 30-40 days, remove the polyethylene cover. Sow the seed with least disturbance to the top soil in the bed.

**Methods of Vegetative Propagation:**

**Budding:**

For budding, the thickness of the rootstock should be near to that of a pencil. It is better to take well swollen and unsprouted buds from leaf axils of mature one-year-old twigs of the scion variety. 'Forkert' or 'patch' methods can be used and the size of the bud wood may be nearly 2x1 cm and budded at a height of nearly 15cm above the ground level. Polythene tape can be used for keeping the buds close to the stock, about 2 weeks after budding the tape can be opened up to examine the success of the budding. After the bud starts sprouting the top portion can be cut. Depending on the type of crop 'T' or 'Inverted T' budding can also be adopted for some of the ornamental crops.

**Grafting:** The common method of grafting is 'inarching' or 'approach grafting'. 'Veneer' grafting has also been found to simple and successful method. For 'Veneer' grafting the scion must be taken from one-month-old shoot duly defoliated for forcing the buds. About 3-5 cm long shoot with one or two buds is used for grafting. The percentage success during July has been recorded as 80%. In places where humidity is more than 70% or where mist chamber facility is there soft wood grafting and stone grafting can be practiced.

**Points to be remembered while grafting:**

1. The scions and rootstock should be preferably of the same diameter (for veneer)
2. The scions should be pre-cured
3. Grafting should be taken up when there is high humidity
4. Grafted plants are to be kept in mist if possible

5. Grafts should be labeled after grafting so that varieties are not mixed
6. Rootstock portion should be cut off after the leaves of the scion turns green.

**Stooling:** This method can be used for quick multiplication of desired varieties. In this method 3-4 years old plants are cut down near to the ground. When the new shoots emerge, IBA (5000ppm) is applied in lanolin in ring during July. After about 10 days soil is earthed up to cover the ringed part. By September, the rooted shoots can be separated. This method is easy and plot of 4-5 m square can yield 300 rooted shoots each year.

**Air layering:**

In this method, limbs of 1/2 inch or more in diameter are girdled by removing a strip of bark about one and half times the thickness of the limb. The girdled area is bound with a ball of moistened sphagnum moss several inches in diameter and 4.5 inches long which is then wrapped with a sheet of polythene paper and tied securely at each end with rubber bands or string. Usually roots begin to form in 3-5 weeks. When the roots grow through the ball of moss, the stem may be severed below the girdled area gradually. The polythene film is then removed from the rooted stem, which is then severed, potted and kept in the shade until new leaves appear. When the new growth is 6-8 inches long the plant can be hardened in full sunlight, preparatory to transplanting in the field.

**Management of plants after propagation:**

The propagated plants need to be hardened. It is always better to harden them in the shade net houses or climate controlled houses. If these are not available then they need to be kept in semi-shade conditions, so that there is no mortality of plants when they are taken to the main field. Timely sprays for insects and diseases need to be given after ascertaining the cause. To maintain the plants in healthy condition, it is better to given micro-nutrient sprays. However, it is also of paramount importance that the plants need to be labeled properly so that the variety is not mixed up.

**Points to be remembered while labeling:**

1. The material taken for labeling should be long lasting
2. Generic or species name should not mentioned
3. Common name of the variety needs to be written without spelling mistake
4. Label should be written with permanent markers

5. If the variety is pre-determined then it is better that printed labels with the name and symbol of the Institute should be mentioned.
6. Self locking labels, which do not come out easily while handling and transport should only be used.
7. For the identification of the plants, labels in local languages can be written in addition to English.

*Annexure-1*

**Standard Propagation Methods for Some Important Tropical and Sub-tropical Fruit Crops**

| *Sl. No.* | *Crop* | *Propagation Methods* |
|---|---|---|
| 1 | Mango | Softwood / Veneer / Epicotyl grafting |
| 2 | Sapota | Softwood grafting |
| 3 | Guava | Patch Budding |
| 4 | Banana | Sword suckers |
| 5 | Pineapple | Suckers, Slips |
| 6 | Citrus fruits | Patch Budding |
| 7 | Custard apple | Wedge grafting |
| 8 | Grape | Wedge grafting / patch budding, rooted cuttings |

**Annexure-2**

**Illustration of the model for Quality Control Programme for Mango**

***A) Step-:. Target Setting:***

Potential area expansion for 'Alphonso' mango during planting season (June – July) of 2009 in zone 'ABC' @ 100 trees per ha : 20,000 ha

Target for mango grafts, ready for planting by May 2009 : 2,40,000 no

Targets for site 'A' : 80,000

Targets for site 'B' : 60,000

Targets for site 'C' : 60,000

Targets for site 'D' : 40,000

Total : 2,40,000 (Includes 20 % extra for discounting field mortality)

***B) Step-2: Flow Chart for Production of 40,000 grafts at site 'D' ('XYZ' Nursery:)***

Target of saleable mango grafts for April 2009 : 40,000 number

Number of rootstock seedlings to be grafted (softwood) during July to October 2008 (assuming 80% graft success) : 50,000 number

Number of seeds to be sown in nursery beds during August 2007 (assuming 50% germination) : 100,000 number

Number of polythene bags to be filled (10% extra) during September 2007 : 55,000 number

***C) Step-3: Inputs required:***

a) Material

| *Item* | *Quantity* | *Approximate cost (Rs)* | *Time of procurement* | *Specifications* |
|---|---|---|---|---|
| Mango stones | 100,000 no | 10, 000 | 25/07/2007 | Eg: Stone – freshly extracted from fully ripe fruits, completely sinking in water, shade dried for three days and treated with 500 ppm carbendazim<br>Scion – 5 to 6 months old, pre-cured, treated with carbendazim 500 ppm |
| Sand | 20,000 Kg | 25,000 | 20/08/2007 | |
| Red Earth | 20,000 Kg | 20,000 | 20/08/2007 | |
| FYM | 35,000 Kg | 45,000 | 20/08/2007 | |
| Super phosphate | 1,200 Kg | 8,500 | 28/08/2007 | |
| Carbendazim | 20 Kg | 12,000 | 20/07/2007 | |
| Polybags 9"x7" | 55,000 no | 24,000 | 20/08/2007 | |
| Alphonso scion | 50,000 no | --------- | March to October, 07 | |

***b) Works:***

| *Item* | *Quantity (no.)* | *Approximate cost (Rs.)* | *Time of start – completion* | *Specifications* |
|---|---|---|---|---|
| 1) Preparation of potting mixture and filling of polythene bags | 55,000 | 22,000 | 01/09/07 - 30/09/07 | 1:1:1 mixture of red earth, FYM & sand, v/v |
| 2) Soft wood grafting | 50,000 | 75,000 | 01/03/07 - 31/ 10/07 | Standard procedure |

***D) Step 4. Verification of specification standards:***

| *Input* | *Specification* | *Quality control Procedure* |
|---|---|---|
| 1) Water | Less than 0.5 $dsm^{-1}$ EC, pH 6.0 –7.0 | Check water monthly with conductivity bridge, pH meter; take corrective steps if needed |
| 2) Media | Harmful / beneficial microbes / nematodes | Colony count 45 days after media preparation |

*Note*: The above given details in this annexure are arbitrary for illustration purpose only and are also not comprehensive.

*Annexure- 3*

**Quality standards set by ICAR for softwood mango grafts**

| *Sl. No.* | *Characters* | *Observations* |
|---|---|---|
| 1 | Method of propagation | Soft wood grafting |
| 2 | Type of Rootstock | Newly emerged stock having bronze coloured leaves |
| 3 | Age of Rootstock | Not more than one year |
| 4 | Diameter of Rootstock (cm) | .20-.35 cm |
| 5 | Age of scion | 3-4 months old having plumpy bud |
| 6 | Diameter of scion | .20-.35 cm |
| 7 | Length of scion | 10-15 cm |
| 8 | Plant height | 75-90 cm |
| 9 | Stem girth | 4-5 cm |
| 10 | Root type / Architecture | 1- Tap root<br>2- Root should not be overgrowth<br>3- Coilage of root should not be there |
| 11 | Uniformity of union | 1- Smooth<br>2- Union of graft 10-12 cm from root stock |
| 12 | Foliage | 1- Healthy<br>2- Green |
| 13 | Disease / pest (Health standard) | 1- No Die back symptom<br>2- No nutritional deficiency<br>3- No attack of leaf eating beetle |
| 14 | Time of grafting | February – December |
| 15 | Precaution | 1- Earth ball should not be cracked and open<br>2- Earth ball should be tightly packed with grasses for transportation<br>3- Watering regularity for long transportation |

*Annexure-4*

**Estimated planting material requirement of major tropical and subtropical fruit crops during the next five-year period, based on the likely area expansion and planting density**

| *Crop* | *Area during 2004 –05 (000'ha)* | *20 % area increase over 2004 –05 area in next 5 years @ 4 % year (ha)* | *Conventional planting density per ha (no)* | *Projected number of planting material needed* |
|---|---|---|---|---|
| Banana | 529.7 | 105940 | 3000 | 317820000 |
| Citrus | 712.4 | 142480 | 300 | 42744000 |
| Grape | 60.2 | 12040 | 1600 | 19264000 |
| Guava | 162.0 | 32400 | 400 | 12960000 |
| Litchi | 60.0 | 12000 | 100 | 1200000 |
| Mango | 1961.9 | 392380 | 100 | 39238000 |
| Pineapple | 81.2 | 16240 | 20000 | 324800000 |
| Pomegranate | 112.5 | 22500 | 625 | 14062500 |
| Sapota | 133.1 | 26620 | 100 | 2662000 |
| Others | 4963.8 | 992760 | 100 - 400 | 99276000 – 397104000 |

*Note*: Area under fruit crops during 2004 – 05 is as per database of National Horticulture Board; Projected number of planting material required for area expansion can go up by 15 to 25 % in the different fruit crops, considering about 10 % field mortality of planting materials and a gradual shift to higher planting densities than the conventional in the new areas. The growth rate projected for cropped area under fruits is uninformed, modest estimate.

*Annexure-5*

**Estimated cost for establishing Hi-tech mango nursery with one lakh graft production capacity per year.**

| *Sl. No.* | *Item* | *Cost (Rs. In lakhs)* |
|---|---|---|
| 1 | Land 1.5 ha | 8.0 |
| 2 | Establishing high density scion bank in ½ ha of above area with drip irrigation | 2.5 |
| 3 | Bore well with pump set and water storage tank | 2.5 |
| 4 | Mist chamber, 200 $m^2$ with foggers automatic controls | 12.0 |
| 5 | Shade net house, 5000 $m^2$ with overhead inverted sprinklers | 5.0 |
| 6 | Low cost polyhouse 3000 $m^2$ | 3.0 |
| 7 | Potting shed 200 $m^2$ | 2.0 |
| 8 | Soil sterilizer with accessories | 3.5 |
| 9 | Store room for inputs, equipments, implements | 4.0 |
| 10 | Quality verification facilities | 1.0 |
| 11 | Misc. (implements, etc.) | 1.0 |
| | **Total** | **44.5** |

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR CITRUS FRUITS

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## 1. Mandarin

| **Institute Name** | NRCC, Nagpur |
|---|---|
| **Name of the Crop** | Mandarin |
| **Botanical Name** | Citrus reticulata |
| **Information regarding mother plants** | Healthy, high yielding with good fruit quality |
| **Method of propagation** | Vegetative |
| **Vegetative propagation** | Budding |
| **Scion preparation** | |
| Age of the scion shoots | 5 to 6 month |
| Preparation of scion shoots/buds | |
| Diameter of scion | 5 to 6 cm |
| **Rootstock preparation** | |
| Name of the rootstock | Rough lemon/ Rangpur lime |
| **Age of the rootstock** | 11 to 12 months |
| **Diameter of the rootstock** | 6 to 8 mm |
| **Rootstock propagation** | By Seed |
| **Standards of the planting material** | Height of the plant : 40 to 60 cm |
| **Height and condition of union** | 9” above ground/ soil surface and smooth union |
| **Scion/ rootstock diameter at the union** | 5 to 6 cm and 6 to 8 cm |
| **Growth of the plant** | Vigorous |
| **Condition of the earth ball** | In polythene bag intact and moist |

**Disease incidence (Fungal, Bacterial, Viral, etc.) :** Plant should be free from Phytophthora, Viruses and Greening diseases

**I Name of disease** – ***Phytophthora* infections**

**Causal organism** – ***Phytophthora nicotiana, palmivora and citrophthora cause damping off of citrus seedlings in nursery***

**Detection and diagnosis –** Isolation of ***Phytophthora*** on selective culture media

**II Name of the disease** - Greening , New Name - Citrus Huanglongbing (HLB)

**Causal organism** - ***Candidatus Liberibactor asiaticus***

**Detection and diagnosis** – i. Indexing

ii. Polymerase chain reaction (PCR)

**III Name of the disease** - Virus and virus like diseases of citrus

**Causal organism** - Citrus Tristeza Virus (CTV), Mosaic and Ringspot

**Detection and diagnosis** – i**. Indexing ii. ELISA**

**ii. Polymerase chain reaction (PCR)**

**Insect pest incidence :** Plant should be free from Lear miner, Aphid, Psylla , Mites and Thrips

**I Causal organism** - **Leaf miner (***Phyllocnistis citrella***)**

**Detection and diagnosis** - Visual

**II Causal organism** **-** Citrus psylla *(Diaphorina citri*)

**Detection and diagnosis** -. Visual.

**III Causal organism** **- Citrus Aphids (***Toxoptera sp.***)**

**Detection and diagnosis** - Visual

**Nutrient deficiency Observed in Nursery**.

**Detection and Diagnosis**- Nitrogen

General yellowing of leaves

**Zinc**

Pointed leaves, smalling of leaves, rosetting and interveinal chlorosis.

## 2. Sweet Orange

| **Name of the crop** | Sweet Orange |
|---|---|
| **Botanical Name** | Citrus sinensis |
| **Information regarding mother plants** | Healthy, high yielding with good fruit quality |
| **Method of propagation** | Vegetative |
| **Vegetative propagation** | Budding |
| **Scion preparation** | Age of the scion shoots : 5 to 6 month |
| | Preparation of scion shoots/buds |
| | Diameter of scion : 5 to 6 cm |
| **Rootstock preparation** | Name of the rootstock : Rangpur lime/ Rough lemon |
| | Age of the rootstock : 11 to 12 months |
| | Diameter of the rootstock : 6 to 8 mm |
| **Rootstock propagation** | Seed |
| **Standards of the planting material** | Height of the plant : 40 to 60 cm |
| | Height and condition of union : 9" above ground/ soil surface and smooth union |
| | Scion/ rootstock diameter at the union : 5 to 6 cm and 6 to 8 cm |
| | Growth of the plant : Vigorous |
| | Condition of the earth ball : In polythene bag intact and moist |

**Disease incidence (Fungal, Bacterial, Viral, etc.) :** Plant should be free from Greening, Phytophthora and Viruses

**I Name of disease** – ***Phytophthora* infections**

**Causal organism** – ***Phytophthora nicotiana, palmivora and citrophthora* cause damping off of citrus seedlings in nursery**

**Detection and diagnosis –** Isolation of ***Phytophthora*** on selective culture media

**II Name of the disease** - Greening , New Name - Citrus Huanglongbing (HLB)

**Causal organism** - ***Candidatus Liberibactor asiaticus***

**Detection and diagnosis** – i. Indexing

ii. Polymerase chain reaction (PCR)

**III Name of the disease** - Virus and virus like diseases of citrus

**Causal organism** - Citrus Tristeza Virus (CTV) and Mosaic

**Detection and diagnosis** – i. **Indexing**

**ii. Polymerase chain reaction (PCR)**

**Insect pest incidence :** Plant should be free from Lear miner, Aphid, Psylla , Mites and Thrips

**I Causal organism** - **Leaf miner (*Phyllocnistis citrella*)**

**Detection and diagnosis** - Visual

**II Causal organism** **-** Citrus psylla *(Diaphorina citri*)

**Detection and diagnosis** -. Visual.

**III Causal organism** **- Citrus Aphids (*Toxoptera sp.*)**

**Detection and diagnosis** - Visual

**Nutrient deficiency Observed in Nursery**.

**Detection and Diagnosis- Nitrogen**

General yellowing of leaves

**Zinc**

Pointed leaves, smalling of leaves, rosetting and interveinal chlorosis.

## 3. Acid Lime

| Name of the Crop | Acid lime |
|---|---|
| **Botanical Name** | Citrus aurantifolia |
| **Information regarding mother plants** | Healthy, high yielding with good fruit quality |
| **Method of propagation** | Seed |
| **Seed propagation** | |
| Germination % | 85-90 % |
| Seed storage | Use fresh seed |
| Seed treatment | Treat the seed with Bavistin before sowing |
| **Standards of the planting material** | |
| **Height of the plant** | 45 cm to 60 cm |
| **Growth of the plant** | Vigorous |
| **Condition of the earth ball** | Intact & moist |

**Disease incidence (Fungal, Bacterial, Viral, etc.) :** Plant should be free from Phytophthora, Viruses and Greening diseases

**I Name of disease – *Phytophthora* infections**

**Causal organism – *Phytophthora nicotiana, palmivora and citrophthora* cause damping off of citrus seedlings in nursery**

**Detection and diagnosis –** Isolation of ***Phytophthora*** on selective culture media

**II Name of the disease -** Greening , New Name - Citrus Huanglongbing (HLB)

**Causal organism -** Citrus canker

**Causal organism -** *Xanthomonas axonopodis pv. citri*

**III Name of the disease -** Virus and virus like diseases of citrus

**Causal organism -** Citrus Tristeza Virus (CTV)

**Detection and diagnosis** – i. **Indexing**

**ii. Polymerase chain reaction (PCR)**

**Insect pest incidence :** Plant should be free from Lear miner, Aphid, Psylla , Mites and Thrips

**I Causal organism - Leaf miner (***Phyllocnistis citrella***)**

**Detection and diagnosis** - Visual

**II Causal organism -** Citrus psylla *(Diaphorina citri*)

**Detection and diagnosis** -. Visual.

**III Causal organism - Citrus Aphids (***Toxoptera sp.***)**

**Detection and diagnosis** - Visual

**Nutrient deficiency Observed in Nursery**.

**Detection and Diagnosis- Nitrogen**

General yellowing of leaves

**Zinc**

Pointed leaves, smalling of leaves, rosetting and interveinal chlorosis.

# GOOD NURSERY PRACTICES TO RAISE DISEASE - FREE PLANTS OF CITRUS

Citrus is one of the largest fruit industry in the world with average production of 104.505 million tones/ year out of total world fruit production (475.504 million tones/year) FAO, 2002. In India it is the third largest fruit industry of the country after mango and banana. India ranks sixth among top citrus producing countries contributing 4.80 % to the world's total citrus production. Still, the production per ha is much lower than other countries like USA, Brazil and Spain etc. Unavailability of disease free planting material of citrus has been the basic reason for such a low production, short productive life and gradual decline of citrus trees. Citrus trees are propagated by seed and by vegetative means. Vegetative propagation is preferred because it ensures true to type plants, uniform quality, regular and early bearing etc. In most areas, a citrus tree is produced by budding the desired scion variety budded into the chosen seedling rootstocks . In process of bud selection , it is required to ensure that clonal purity, good physiological vigour and yield potential are maintained and bud material should be free from transmissible diseases like viruses/GB. Seedlings are being used for limes and mandarin to a certain extent in North Eastern Hill Region and Coorg Region of India (Ghosh and Singh, 1993 ). In case of lemons and sweet lime, air layering and even cutting are being employed for raising plants. Citrus is highly susceptible to both biotic and a biotic stresses but virus and virus- like pathogens are the main biotic agents responsible for poor tree health and reduced yield ( Ahlawat and Pani, 1999 ). For growing healthy citrus trees it is essential to have mandatory budwood certification programme. Unless the orchards are planted with disease-free nursery stock, none of the potential of the improved practices can be fully realised. Therefore production of disease-free planting material should made mandatory for bright and healthy future citrus industry of India. Various aspects to be considered for raising healthy disease-free planting material of citrus.

NRC for Citrus, Nagpur has taken a mission oriented programme on production of disease free planting material of Nagpur mandarin, acid lime and sweet orange (Mosambi) by adopting the most advanced and internationally accepted techniques of nursery management duly standardized at the Centre (Singh *et a*l.) The disease free (from virus and fungal diseases) plants were raised to supply to Government owned nurseries for raising mother plants and to the citrus growers to overcome the problem of citrus decline. The techniques adopted in production of disease free planting material are briefed here (Singh, 1999).

## CONTAINERISED NURSERY SYSTEM

In India most of the citrus nursery are grown as field nursery. In field nurseries, the eradication of soil borne pathogens like *Phytophthora* once introduced becomes very difficult. To avoid this problem, concept of containerised nursery system was adopted. The infrastructure required for such nurseries includes shade net houses (50 % shade), sterilised plastic trays, UV stabilised black polybags (100 **µ),** UV stabilised transparent polythene for solarization, fumigation of potting mixture, a separate set of nursery equipments etc.

**Potting mixture:** The potting mixture, consisting one part of virgin fertile soil + sand + FYM (sterilized), was used in plastic trays for seed sowing in primary nursery. The same sterilized mixture was used for filling the polybags to be used in secondary nursery.

A. **Soil solarization:** The potting mixture was first collected on a concrete floor and spread in the form of flat bed of 1.5' thick layer. These beds were completely drenched with water before covering it with 100 µ UV stabilized transparent polythene sheets in summer months (April- May) when atmospheric temperature goes up to 45 – 46 $^0$C. The edges of polythene sheet were completely sealed with soil to avoid vapour loss, which allowed the inside temperature to rise upto 54$^o$C. Soil solarization was done for 4 – 6 weeks.

B. **Soil fumigation:** The solarised soil was further fumigated with Basamid (Dazomet) granules, a soil fumigant, which releases methyl isocynide gas and thereby, completely eliminates *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. from the soil. Solarised and fumigated potting mixture was used to fill trays and bags.

**C. Steam sterilisation**

This is one of the quickest method of potting mixture sterilisation In this method steam are passed through potting mixture which kill all pathogen and weeds. First mixture were filled in jeep trolly which have nozzel in bottom of trolly. Then steam which is generated in boiler passed in this covered trolly for about 20 minute.

## Selection of seed :

Seed should be collected from healthy fruits of recommended cultivars / rootstocks. Only selected trees free from diseases be used as seed source. Fruit that has fallen is more subject to brown rot infection . Seed in rotten fruit may be invaded by fungi that later contaminate the seedling. Seeds are extracted from fully ripened fruits by making a shallow cut through the rind round the centre of the fruit and twisting the two halves of fruit apart . Seeds are then washed into cold water with rubbing in ash to make tree from pulp and dried under shade condition. The sound seeds, being of greater density are separated from the underdeveloped seeds. The number of seeds per fruit varies in citrus species and cultivars. Sharma (1982 ) recorded the highest number of seeds per fruit in Karna Khatta (34), followed by Jatti Khatti (31), Carrizo (23), Rangpur lime (19.5), Cleopatra (15), Savage (9) and pectinifera (5).

The seeds of most citrus spp. are recalcitrant. Their viability during storage varies depending upon species and storage conditions. Serious loss of seedlings in the seed bed and nursery from infection of fungi *Phytophthora* have been reported. Therefore seed should be treated with a fungicide like Bavistin to reduce the infection. Seeds should be

sown as early as possible after extraction , since citrus seeds give the highest germination if planted immediately after extraction.

## Role of rootstock :

In our county citrus trees are propagated by vegetative as well as by seeds. Propagation by budding should be encouraged particularly for sweet orange and mandarin. Since budded plants come early in bearing (4-5$^{th}$ year) than seedling plants (9-10 years). Using rootstock exert profound influence on the vigour, productivity, quality of fruits, longevity of the scion, adaptability to soil climatic conditions , etc and also response to different pest and diseases. It is therefore, important to appreciate the need for using the appropriate rootstock suiting to a particular location so that the threat of diaback can be minimized. The selection of proper rootstocks for different regions is quite complicated and required serious attention. However in our country rough lemon and Rangpur lime are commercially used as rootstock for different mandarin and sweet orange cultivars.

## Raising of seedling in primary nursery :

For raising disease- free nursery it is required to grow primary nursery in tray. Since most of the citrus nurseries are reported to be infected with *Phytophthora* ). Plastic tray 60x40x12 cm size was found ideal for primary nursery. After making 6 holes in buttom of trays to drain out excess water, trays are filled with sterilised potting mixture (One part fertile soil + one part sand + one part FYM) and trays are kept at 1.5 feet height from the ground on the bricks or on cement platform to check the contamination. After leveling the mixture upto top level of trays. Seeds are sown at depth of 1- 1..5 cm with spacing 2.5 to 3.0cm in the row and after sowing light shower irrigation should be done with a water can. The germination start within 20-25 days after seed sowing depending upon the rootstocks. Citrus seeds usually germinate at a soil temperature above 55 $^0$ F. The optimum temperature is between 80 and 90 $^o$ F. It is necessary to protect the primary nursery with some type of shelter . Both intense sun and wind may cause emerging seedlings to burn. The shade net (50 % shade) found ideal for citrus seedlings. Irrigation of seedlings is most critical aspect. Germinating seeds are quickly killed by drought and on other hand , excessive moisture favours development of damping - off fungus. Therefore frequent and light watering is required for newly planted seed beds.

## Transplanting of seedling in secondary nursery :

Seedling when 4 to 6” tall having 8 to 10 leaves are transplanted to black polythene bag of 12” to 6” size having 3-4 holes at the bottom to drain out excess water. The polythene bags are filled with sterilized potting mixture and arranged in shade net. To ensure uniform nucellar seedlings, discard of markedly smaller or too taller ones at the time of transplanting. Seedlings from primary nursery (Trays) should be uprooted with fork carefully to minimise root damage. The hook-necked bent or twisted taproot seedlings should be avoided. Selected seedling should be treated with Ridomil (2.75g / litre water ) solution before transplanting to check the contamination at the time of transplanting. The seedlings are transplanted in the month of July-Aug after commencement of monsoon. The holes in which the seedlings are to be planted are usually made by stick. The roots of the seedling are then placed in the opening and

pressing the soil firmly around the seedling to make contact of soil with roots for quick and better establishment. Irrigation should follow the planting of seedlings. In the beginning water should be applied frequently to overcome the shock of transplanting. The nursery must be kept free from weeds at all times. The side shoots should be removed regularly to develop single straight stem. Frequent and light irrigation should be given as per need taking care to avoid water stagnation in polythene bags. If such bags found excess water may drain out through making the new holes in polythene bags.

## Selection of mother plants and budwood :

Selection of mother plants for budwood is the most critical parts of production of disease-free planting material. Since plant productivity, longevity, fruit quality and most important free from diseases all depend on types of mother plants. Therefore mother plants should be selected from authentic sources with known pedigree in respect to health, vigour , regular bearing and high yield with good quality fruit. Selected plants should be indexed against diseases (viruses and Greening bacterium) and only disease-free plants should be used as bud source. Such disease-free plants should be marked and budstick should be carefully taken to avoid infection. For this purpose knife used should be disinfected with alcohal or sodium hypochlorite solution. Bud wood should be always taken from fairly well matured wood of current season growth or next to last growth. Round twigs having longitudinal white streak on the bark, swellen buds ready to grow should be selected. First one or two basal buds, which are usually somewhat imperfect should be discarded. Bud wood should be kept in moist sphagnum moss and gunny bag to avoid exposure desiccation.

### DIAGNOSIS OF VIRUS/VIRUS LIKE DISEASES

The virus detection tests were performed for the disease status of mother plants.

- More than fifteen virus and virus like pathogens have been reported to attack citrus in India.
- Samples were taken from these identified elite mother plants for biological/serological detection against major pathogens *viz.*, citrus tristeza virus, citrus mosaic, citrus ringspot, citrus exocortis, viroid and greening bacterium.

**i. Serological diagnosis**:

Serological indexing was done in DAS-ELISA by using monoclonal (CTV) and polyclonal (CTV, RS, mosaic) antibodies.

**ii. Bo-diagnosis:**

The biological indexing was also performed simultaneously using indicator plants like acid lime for tristeza, sweet orange for greening bacterium and mosaic virus, Etrong citron for exocortis and sweet orange and *Chenopodium quinoa* for ring spot etc. under insect proof controlled conditions.

Thus, the virus free plants among selected elite mother plants were identified for further multiplication.

## Budding and maintenance of budlings

Budding should be done in the season when bark would slip. The time of budding effects bud take (Table 1) and subsequent growth of plants. Mukherjee *et al*. (1966) observed that October was the best time for budding pineapple sweet orange on *C. jambhiri* rootstock under Delhi condition. In Vidarbha region of Maharashtra budding is usually done in Nov- Jan when seedling attain the girth of 3.0 to 3.5cm at 9" height from ground level following the 'T' or shield budding method. The budded portion should be wrapped with 100 guage polythene strips of 1.2-1.8 cm wide.

**Table 1 : Best time of budding of citrus in different part of country**

| Scion/rootstock | Best time | Place | Reference |
|---|---|---|---|
| **Sweet oranges** | | | |
| Pineapple/ C. jambhiri | October | Delhi | Mukherjee *et al*.(1966) |
| Mosambi / C. Karra | February | Allahabad (UP) | Srivastava and Arya (1969) |
| Jaffa/ Jatti Katti | Feb-March or Sep- October | Ludhiana (Punjab) | Nijjar (1980) |
| Malta/ *C. karna* | February and September | Udaipur (Rajasthan | Sen and Kapadia (1984) |
| **Mandarins** | | | |
| Kinnow/ *C. jambhiri* | May | Palampur (HP) | Joolka (1986) |
| Nagpur/ *C.jambhiri* and *C.limonia* | Mid - December | Akola (Maharashtra) | Rakhonde and Tayde (1987) |
| Sangtra/ *C. karna* | February and September | Udaipur (Rajasthan) | Sen and Kapadia (1984) |

The tendency has been to bud at a low height of 4 to 6" with the result after few year bud union gets buried in the soil and becomes prune to diseases. It will be better to bud at a height of 9" above the grounds level so that a tree develops good productive

canopy above ground without being affected by diseases and injury. Polythene strips wrapped on budded portion should be removed after 14- 21 days for better development of bud sprouts and examined to determine their condition. The rootstock stem at 2-4" above the bud union must be prunned 2-3 times and all side shoot should be removed. If the bud remains green for longer period, heading back, lopping or bending the rootstock above the budding point forces bud sprouting. The budlings are provided with frequent light irrigation and Urea twice in a month to get good material ready for transplanting in July- Aug. Nursery must be kept free from weeds and water stagnation The side shoots below the bud union should be removed from time to time.

## Plant protection measures

In citrus nurseries, *Phytophthora* diseases may appear any time of plant growth through contaminated water, soil and even through nursery workers and implements.Therefore a regular monitoring should be done for *Phytophthora* infection In case of infection, the infected/contaminated plants should immediately uprooted and destroyed to keep the nursery totally free from *Phytophthora* and other diseases.

The nursery plants are sprayed with Bavistin @ 1 g/lit water at monthly interval as a prophylactic measure. If plants affected by *Phytophthora* spp. are noticed, remove the affected plants with polythene bags and spray Ridomil MZ 72 @ 2.75 g OR Aliette @ 2.5 g/lit. Nursery implements should be disinfected regularly with sodium hypochloride solution and at the entry of nursery. The arrangement should made to disinfect the shoe of workers and visitors with copper sulphate and lime dust. Floor should be regularly sprayed with copper fungicides and at the entrance of nursery. The insect pests in nursery should be managed with the regular application of recommended insecticides

- Monocrotophos @ 1.0 ml /lit. water against leaf miner
- Confidor @ 0.5 ml/lit. water against leaf eating caterpillar/ Thrips
- Nuvan @ 1.5 ml/ lit. water against leaf miner
- Quinalphos @ 1.0 ml/lit. water against aphids
- Dicofol (kelthane) @ 1.5 ml/lit. water or wettable sulphur @ 3 g/lit. water against mites
- Neem oil (1 %) spray when rotated with foliar sprays of any of the above pesticides gave better results against insect pests.

## Tips for raising disease- free nursery

- Nursery site should be away from the citrus orchards.
- Nursery should be raised in containers (plastic trays/polythene bags)
- Only sterilized potting mixture should be used in primary and secondary nursery.
- Always use fresh seeds extracted from healthy fruits and sow in trays under shade conditions for better germination
- Seed trays must be kept at least 1.5- 2.0' above the ground to avoid soil borne contamination.
- Nursery floor should be covered with stones/ stone dust to avoid contamination from soil.

- Only nucellar seedlings should be selected for further growth.
- Seedlings with bent and twisted tap root system should be discarded
- Too long taproot should be cut to ensure the straight penetration of root in soil.
- Seedlings should be transplanted during rainy season/cloudy days in polythene bags for greater survival.
- Seedlings should be treated with Ridomil @ 2.75g and Bavistin @ 1g/lit water at the time of transplanting in secondary nursery.
- Budwood should be taken from disease free selected and certified elite mother plants of known pedigree.
- High budding not less than 9” of height should be done.
- Sterilized knife with alcohol or sodium hypochloride should be used for budding and it should regularly be washed with surface disinfectant.
- Budwood should be selected from fairly well mature non-bearing shoots of current year growth from selected plants.
- Selected mother plants should be monitored regularly for diseases.
- Regular recommended plant protection measures should be followed to control insect pests.
- Prophylactic measures should be taken against diseases and diseased plants should be destroyed.
- Set of nursery implements and workers should be separate.
- **The entry of visitors should be restricted in disease- free area of nursery to avoid contamination.**
- The arrangement should be made to disinfect the shoe of workers and visitors with copper sulphate and lime dust at the entry of nursery.

# SEED AND PLANTING MATERIAL TESTING MANUAL
# FOR
# BANANA AND PLANTAIN


**Prepared by**

**Director**

**NATIONAL RESEARCH CENTRE FOR BANANA**

**TRICHY**

"SEED AND PLANTING MATERIAL TESTING MANUAL FOR BANANA AND PLANTAIN"

| Name of the crop | Banana and Plantain |
|---|---|
| Botanical Name | *Musa spp.* |
| Information regarding mother plants | The mother plants should be true to type, healthy, high yielding, free from viral (BBTV, BSV, BBMV and CMV), fungal (Fusarium wilt) and bacterial (Erwinia/Tip over) diseases and nematodes and corm weevil infestation. |
| Method of Propagation | Vegetative propagation by Suckers |
| a). Planting material standards for propagation by suckers | • 'Sword Suckers' having broad corm with narrow leaves are recommended.<br>• The suckers should be 3 months old, uniform in size, weighing 1-1.5 kg for *Rasthali, Poovan Nendran, Ney Poovan,* and *Cavendish* banana varieties.<br>• For varieties like *Karpuravalli, Red Banana and Monthan,* 4 months old, uniform suckers weighing 1.5-2.0 kg should be used. |
| b). Tissue culture plants | |
| Standards of the planting material for propagation by tissue culture | • The secondary hardened plant should be of true to type, > 30 cm in height, 5 cm in girth with at least five fully opened, healthy, green leaves. The randomly tested plants should have healthy root system and free from nematode, Erwinia and F oC infestation.<br>• Plants exhibiting symptoms of somaclonal variations of unusual foliage characteristics should be discarded. |
| Disease incidence | |
| a) Name of the disease | Panama Wilt/ Fusarium Wilt disease |
| Causal organism<br>Detection and Diagnosis | *Fusarium oxysporum* f.sp. *cubense (FoC)*<br>Suspected corm should be cut and seen for vascular discolouration for FoC infection. |
| b). Erwinia Rot | |
| Causal organism<br>Detection and Diagnosis | *Erwinia carotovora* var. Carotovora and *E. Chrysanthemi*<br>Visual symptoms of dying of the central core<br>Bacterial oozing from the cut stem position. |

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR CASHEWNUT

**Prepared by**

**Director**

**NATIONAL RESEARCH CENTRE FOR CASHEW**

**PUTTUR, KERNATAKA**

## LABELS TO BE USED FOR PREPARING "SEED AND PLANTING MATERIAL TESTING MANUAL"

| Name of the crop | **CASHEWNUT** |
|---|---|
| Botanical Name | *Anacardium occidentale L.* |
| Information regarding mother plants | High yielding, true to type mother plant |
| Method of Propagation | |
| Seed Propagation | Not applicable |
| Vegetative propagation | Grafting (Soft wood grafting) |
| Age of scion shoots/ buds | 3-5 month old |
| Preparation of scion shoots/buds: | 10-12 cm long, precured (1 week in advance), pencil thickness, current season lateral shoot |
| Diameter of scion / size of bud | 1 cm |
| Rootstock preparation | |
| Name of the rootstock | Seedling |
| Age of the rootstock: | 45 to 60 days (25-30 cm height) |
| Diameter of the rootstock | 1 cm at grafting height |
| Rootstock propagation | By Seed |
| Standards of the planting material | |
| Height of the plant: | >30 cm |
| Height and condition of the union | 15-20 cm from collar region |
| Root system of the plant | Well developed |
| Scion and root diameters at the union | 1 cm |
| Growth of the plant | Healthy and erect growing |
| Condition of the earth ball | Good and intact |
| Disease incidence (Fungal, Bacterial, Viral etc | Nil |
| Insect pest incidence | |
| 1. Tea Mosquito Bug (TMB) (*Helopeltis antonii* Sign) | |
| Nature of damage | Sap from tender shoots is sucked by nymphs and adults |

| | |
|---|---|
| Detection and diagnosis | Formation of necrotic lesion around point of stylet insertion by the bug. Lesions on shoots coalise to result in shoot blight |
| 2. Leaf miner *(Aerocercops cyngramma* M) | |
| Nature of damage | The caterpillars mine and feed below epiderimal layer of tender leaves. |
| Detection and diagnosis | Extensive leaf blisters which later dry up, causing distortion, browning and curling of leaves. As the attacked leaf ages, the holes are formed due to drying out of the damaged portion. |

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR GRAPES

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**PUNE, MAHARASHTRA**

LABELS TO BE USED FOR PREPARING "SEED AND PLANTING MATERIAL TESTING MANUAL"

For fruit crop

| Name of the crop | Grape |
|---|---|
| Botanical name | *Vitis vinifera* |
| Information regarding mother plant | All varieties of grape belongs to the family *Vitaceae* and the genus *Vitis*. There are two sub genera in the genus *Vitis, viz. Euvitis and Muscadinia*. All the commercially important varieties of grape belong to sub-genus *Euvitis*. The most important species of Euvitis is *Vitis vinifera.* More than 90 percent of cultivated grape varieties belong to this species. |
| Method of propagation | Cultivars of grapes by and large like many other fruits could be reproduced or propagated asexually. In grapes, two types of plants are being used i.e., rootstock and commercial varieties. Most grapevines including rootstock are reproduced by cuttings (semi hardwood, hardwood and softwood) or by grafting and budding. These methods of asexual propagation ensure that the plants are genetically identical to their parents. It helps in producing the plants in short duration. |
| Seed propagation | Grape is highly heterozygous plant. Studies have shown that the grape seeds have dormancy and therefore do not germinate readily when the freshly extracted seeds are sown in the germination media. Seed propagation is usually practiced for hybrids and in wild varieties. This is not a commercial practice in commercial varieties. |
| **Seed storage** | Seed storage is required only when the seeds of the breeding material are to be preserved. However the cuttings can be stored at 3-4$^0$C. |
| **Seed treatment** | The cuttings of either commercial varieties or of rootstock are treated with 1% Carbendazim solution to disinfect all kind of diseases present. |
| Vegetative Propagation | Cutting/Layering |
| **Age of shoot/buds and diameter of scion/size of bud** | The buds and shoots selected from the mother plant of either rootstock or from the table and wine grapes should be matured. The selected bud should be bold enough while the shoot should be of brown colour indicating its maturity. The cuttings selected for planting in the nursery should be of 0.8- 1.0 cm in diameter. |
| Grafting/Budding | |
| Scion Preparation | |

| **Age of scion shoot/bud** | **Like many other fruit trees, grapevine can also be propagated by grafting. Generally *in-situ* wedge-grafting method is followed. The scion cuttings selected should be one season old and matured having better pith.** |
|---|---|
| **Preparation of scion shoot/buds and its diameter** | The scion cuttings should preferably be selected from the middle portion of the straight canes on the vine. The scion material selected should be from healthy, disease and insect free vine. It should be fully matured with diameter of at least 0.8 cm. Usually the diameter of scion should match with that of rootstock shoot. |
| **Rootstock preparation** | |
| **Name of the rootstock** | In grape cultivation the rootstocks, 1103 Paulsen, 99 Richter and 110 Richter are used against shortage of irrigation water, Salt Creek as salinity tolerant and Dog Ridge for yield and quality. |
| **Age and diameter of the rootstock** | To achieve the graftable size of the rootstock cuttings, the rootstock plants should be of 6-8 months age having 80-100 cm length.<br>For better graft success, the rootstock cuttings should have enough stored food material. Usually 0.8-1.0 cm thick shoots are preferred for better success. |
| Rootstock propagation | The rootstock is propagated by hardwood cuttings. |
| Standards of planting material | |
| **Height of the plant and height and condition of the union** | While *in-situ* grafting of any commercial variety, the plant height is not considered but the height of the shoot at which grafting is done should be of 0.8-1.0 cm thickness. The grafting of commercial variety is done on the rootstock at 30 to 45 cm height from the ground. |
| **Scion and rootstock diameter at the union** | For better graft success, the cuttings selected for grafting and the rootstock on which the grafting is to be performed should be of 0.8-1.0 cm diameter. |
| **Growth of the plant** | Once the grafted plant sprouts, the successive growth should be encouraged with timely irrigation and removal of suckers from rootstock region. |
| **Root system of the plant and condition of the earth ball** | In case of grafting, the root system of the rootstock is already well developed as the planting was done 6-8 month back in the field. At this time the length of primary roots will be of 30-35 cm. While planting the rooted cuttings of the rootstock, earth ball should be intact and excess root system can be trimmed off. |
| Preservation of seed/ planting material | In grapes, many advanced techniques are being used for storage of buds and the cuttings. The scooped buds required for budding in failed plants of rootstock and cuttings for multiplication are stored at 3-4$^0$C. The buds and the cuttings are taken out only at the time of |

| | |
|---|---|
| | budding or planting. |
| Disease incidence (Fungal, Bacterial, Viral, etc) | On the rootstock plants diseases are not noticed. However, rust is occasionally observed during rainy season. In case of wine grapes and newly introduced rootstock/varieties, viral diseases like fan leaf virus, leaf roll virus, etc. may be a problem. Detection of viral particle through ELISA kits may helps in eliminating virus-infected plants. |
| | Detection and diagnosis |
| Insect pest incidence | Causal organism<br>On rootstock mainly chaffer beetle is seen during the rainy season. However on the table grapes thrips, jassids and mealy bugs are problem. |
| | Nature of damage: Thrips suck the cell sap resulting in to scars on the berry. The mealy bugs are also sucking the sap from the berries and puts white secretion making the grapes unfit for eating. Chaffer beetle pest eats the leaf and makes small cuts of different shapes. |
| Nematode incidence | The rootstocks 1613 C, 140 RU, Salt Creek, Teleki 5C and 99 R are used against tolerance to nematodes. |
| Nutrient deficiency | The rootstock plants do not show nutrient deficiency to a large extent. However, on the younger leaves, ferrous deficiency is observed which can be corrected by spraying or soil application of ferrous Sulphate. In table grapes, the deficiency symptoms of potash, magnesium, zinc, boron, etc is observed. |

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR LITCHI

**Prepared by**

**Director**

**NATIONAL RESEARCH CENTRE FOR LITCHI**

**MUJAFFARPUR, BIHAR**

# Seed and Planting Material Testing Manual for litchi

Name of the crop : Litchi

Botanical name : *Litchi chinensis* Sonn.

Information regarding parent material

**Age of the elite mother tree** : >10 years and above, should be a stable yielder and free from pest and diseases.

**Type of planting material used for propagation** : Vegetative shoot

**Method of propagation** : Vegetative (air layering)

**Age of the shoot** : > 10-12 months

**Diameter of the shoot** : 1.0-1.25 cm

**Length of the shoot** : 25-35 cm

**Age of the rooted cutting** : 2.5-3 months from date of air layering and planting in **the nursery bed**

**Height of the air layered plants** : 30-45cm

**Diameter of the air layered plants** : 1.5-2 cm

**Growth of the plants** : Vigorous

**Root system** : Well developed

**Condition of the earth ball** : Intact and moist

**Varietal purity** : Varietal purity must be maintained

**Insect- pests observed in nursery**

1. Leaf miner ( *Acrocercops hierocosma* Meyr)

   Detection and diagnosis :Visual; larvae damage the leaves of new flush; bore and mine in the mid rib of leaves making tunnel

2. Litchi mite (*Acerya litchi* Keifer)

   Detection and diagnosis Visual; presence of chocolate velvety brown growth on ventral surface of leaves, tender shoots

3. Bark eating cater pillar (*lndarbela teraonis* Moore)

   Detection and diagnosis : Visual; presence of holes at the joints of branches and covering of silk, wood fragments and excreta, common on older trees

4. Shoot borer (*Clumesia transversa)*

   Detection and diagnosis : Visual; yellowing and wilting of leaves due to damage of

conducting vessels, common on young flush

5. Leaf roller (*Platypeplus aprobola)*

   Detection and diagnosis : Visual; longitudinal roll of tender leaves together and feeding

6. Leaf cutting weevil (*Millocerus spp)*

   Detection and diagnosis : Visual; cutting of leaves from margin

**Disease incidence observed in nursery**

1. Name of disease : Anthracnose disease

   Causal organism : *Botriodiplodia theobromae*

   Detection and diagnosis : Visual; appearance of brown to black irregular spot on the new leaf

2. Name of disease : Leaf spot disease

   Causal organism : *Pestalotia pauciseta*

   Detection and diagnosis : Visual; appearance of circular brown to black irregular spot on leaf

3. Name of disease : Dieback disease :

   Causal organism : *Diplodia spp*

   Detection and diagnosis : Visual; appearance of brown lesion on shoots

**Nutrient deficiency observed in nursery**

**Detection and Diagnosis**

N : Yellowing of leaves and stunted growth

K : Leaf tip drying extending to leaf margin

Cu : Reverse cup shaped, narrow elongated, abnormal leathery leaf growth ppearance on young leaves

Zn : Drying of leaf from margins all along.

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR POMEGRANATE

**Prepared by**

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**NATIONAL RESEARCH CENTRE FOR POMEGRANATE**

**SHOLAPUR, MAHARASHTRA**

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR POMEGRANATE

| Name of the crop | Pomegranate |
|---|---|
| Botanical Name | *Punica granatum* L. |
| Information regarding mother plants | Free from diseases and Insect-Pests with good bearing habit |
| Method of Propagation | Seed / Divisions / Cutting / Layering / Budding / |
| | Grafting |
| Seed propagation | |
| Germination percentage | 75-90% |
| Seed storage | 6 months to 1 year |
| Seed treatment | Nil |
| Vegetative propagation | Cutting / Layering |
| Age of shoots | 6-18 Months |
| Diameter of shoots | 0.8-1.2 cm |
| Standards of the planting material | |
| Height of the plant | 50 -75 cm |
| Growth of the plant | Erect without suckers |
| Root system of the plant | Fully developed root system |
| Condition of the earth ball | Compact and intact |
| **Disease incidence (Fungal, Bacterial, Viral, etc.)** | |
| **A. Fungal diseases** | |
| Name of the disease | Leaf and Fruit spots |
| i) Casual organism | *Cercospora punicae* |
| **Nature of damage** | Infects leaves and fruits, thereby, reducing yield and affecting fruit quality. |
| **Detection and diagnosis** | The disease is detected on leaves and fruits in the form of dark black circular to irregular spots with slightly raised edges. The disease can be diagnosed through microscopic examination and cultural studies as the pathogen produces conidia. |

| | |
|---|---|
| ii) Casual organism | *Colletotrichum gloeosporioides* |
| **Nature of damage:** | Disease affects leaves and fruits and thereby affecting both quality and quantity of pomegranate. |
| **Detection and diagnosis** | The disease is detected on leaves and fruits as light brown centre and dark brown edges. The spots enlarge with progress of time and coalesce. Exact diagnosis of the disease can be done through microscopic and cultural studies as the pathogen produces elongated hyaline conidia with round ends. |
| (iii)Casual organism | *Alternaria alternata* |
| **Nature of damage** | Affects leaves, stems and fruits, affects fruit quality and reduces yield. |
| **Detection and diagnosis** | Disease appears on leaves, fruits and stems. On fruits numerous black spots appear which may coalesce. Pathogen can be detected under the microscope and through cultural studies as pathogen produces conidia which are ellipsoid with tapering beak and with both transverse and horizontal septa. |
| (iv) **Casual organism** | *Drechslera rostrata* |
| **Nature. of damage:** | Affects leaves and fruits reducing yield and market value. |
| **Detection and diagnosis:** | Disease is observed as minute brownish black spots on leaves and fruits. Pathogen which produces pale to dark olivaceous brown, cylindrical, less curved conidia can be detected through microscopic and cultural studies. |
| Name of Disease | Pomegranate wilt |
| **Casual organism** | *Ceratocystis jimbriata, Fusarium* sp. |
| **Nature of damage** | Disease kills the entire plant thereby resulting in 100% loss. |
| **Detection and diagnosis** | Initially one or two branches of a tree reveal wilt and within few months entire tree gets wilted and killed. The disease can be diagnosed by observing the spores ofthe pathogen. |
| B. **Bacterial diseases** | |
| **Name of Disease** | **Bacterial blight (Oily spot)** |
| **Casual organism:** | *Xanthomonas axonopodis* pv. *punicae.* |
| **Nature of damage** | Disease affects all plant parts. Main damage is observed on fruits which reveal black spots and normally split resulting in enormous losses. |
| **Detection and diagnosis:** | The disease manifest in the form of greyish black spots |

| | |
|---|---|
| | on the leaves and fruits. infected fruits normally split. infected stems reveal girdling and break of at the point of infection. The pathogen can be detected through ooze test under the microscope and identified through cultural studies. |
| **Insect pest incidence** | |
| 1. Fruit borer *(Deudorix isocrates)* | |
| **Nature of damage** | Larvae of the butterfly bore into the fruits and damages the pulp thereby rendering fruit unfit for consumption and market. |
| **Detection and Diagnosis** | Affected fruits reveal holes produced by the larvae of the butterfly. The main infection is observed during the rainy season. |
| **2. Bark eating caterpillar** *(Inderbela quadrinotata)* | |
| **Nature of damage:** | Larva feeds on main trunk and bark on a result tree may dry up and bear no fruit. |
| **Detection and diagnosis:** | Affected parts of the tree reveal excreta and chewed material out of the surface. Insect can be diagnosed by characteristic caterpillars. |
| 3. Fruit sucking moth. *(Achaea janata)* | |
| **Nature of damage** | Caterpillars feed voraciously on leaves and may completely defoliate the tree causing considerable damage. |
| **Detection and Diagnosis** | Moths are nocturnal and suck the juice of fruits. Caterpillars are semiloopers. |
| 4. Mealy bugs *(Ferrisia virgata)* | |
| **Nature of damage** | Nymphs of the insect mainly suck sap from the leaves ,twigs, flowers and fruits thereby reducing fruit quality. Severe infestations result in fruit drop. |
| **Detection and diagnosis:** | Nymphs mainly damage flowers and fruits by forming encrustations. Mealy bugs also secret honey dew favouring sooty molds. |
| 5.Casual organism: Aphids *(Aphis punicae)* white fly *(Siphoninus jinitimus)* Thrips *(Rhipiphorothrips creuentatus)* | |
| **Nature of damage:** | Aphids and whiteflies suck sap from leaves and twigs rendering affected plant parts discoloured and disfigured. |
| **Detection and diagnosis:** | Affected plants may reveal curling of leaves. Thrips infestation may produce conspicuous scares on fruits. |
| Nematode incidence | Nil |
| Nutrient deficiency / Disorders | |
| 1. Internal break down of arils: | |

| Causes: | Complex (Exact cause to be established) Delayed harvesting, Varietal character, Nutritional deficiency etc. |
|---|---|
| Nature of damage | Mainly affects fruits which reveal blackening and rotting of arils only when cut and opened and become unfit for consumption and damage. |
| Detection and diagnosis | Mainly affects fruits which appear healthy but when cut, reveal disco loured , rotten and shrivelled arils. Studies on diagnosing the disease are inconclusive. |
| 2. Fruit cracking: | |
| Causes | (i) Improper irrigation (ii) Boron deficiency |
| Nature of damage | Fruits reveal cracking as a result pulp gets exposed to secondary pathogens, thereby affecting fruit quality. |
| Detection and diagnosis: | Affected fruits reveal severe cracking which may be attributed to improper irrigation or Boron deficiency. |
| 3. Sun Scald: | |
| Causes | Fruits on trees mainly facing direct sun rays reveal sun scald. Improper pruning is one of he reasons. |
| Nature of damage: | Peel of affected fruits reveal sun Burn symptoms reducing market value of such fruits. |
| Detection and diagnosis | Affected fruits reveal sun bum particularly on the fruit surface |

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR ARID FRUIT CROPS

**Prepared by**

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**BIKANER, RAJAASTHAN**

**Manual for seed and planting material of Arid Fruit Crops**
Director, CIAH, Bikaner

| Name of the crop | Ber | Khejri | Date Palm | Lasoda |
|---|---|---|---|---|
| **Botanical Name** | *Ziziphus mauritiana* Lamk | *Prosopis cineraria* | *Phoenix dactylifera* | *Cordia myxa* |
| **Information regarding mother plants** | Mother plants of different cultivars planted in nursery | Identified Mother plant | Identified Mother plant | Identified Mother plant |
| **Method of Propagation** | | | | |
| Seed/ Divisions/ Cutting/ Layering/ /Grafting | Vegetative propagation | Vegetative propagation | Vegetative propagation | Seed |
| **Seed propagation** | | | | |
| Germination percentage | - | - | - | 70% |
| Seed storage | - | - | - | Freshly harvested |
| Seed treatment | - | - | - | Thiram |
| **Vegetative propagation** | | | | |
| **Cutting/Layering** | | | | |
| Age of shoots | - | - | - | |
| Diameter of shoots | - | - | - | - |
| **Grafting/ Budding** | Budding | Budding | Sucker/ offshoots | |
| **Scion preparation** | | | | |
| Age of scion shoot/buds | 50-60days | 4-6 month | 1-1.5 year | - |
| Preparation of scion shoots/buds | Shield | | | |
| Diameter of scion/size of bud | 1.0cm | 1.0cm | - | |
| **Rootstock preparation** | | | | |
| Name of the Rootstock : Seedling/Clonal | Seedling rootstocks of *Ziziphus mauritiana* var | Seedling rootstocks of *Prosopis cineraria* | - | - |

| | rotundifolia | | | |
|---|---|---|---|---|
| Age of the rootstock | 4-6 month | 6-8 month | - | - |
| Diameter of the rootstock | 5mm | 3-4 mm | - | - |
| **Rootstock propagation** : Seed/Clonal | seed | seed | - | - |
| **Standards of the planting material**<br>Height of the plant | 30-35 cm | 25-30 cm | 60-75 cm | 30-35 cm |
| Height of the union | 10-15 cm | 8-10 cm | - | - |
| Scion and shoot diameters at the union | 4-5mm | 3 -4 mm | - | - |
| Growth of the plant | 30-35 cm | 25-30 cm | 60-75 cm | 30-35cm |
| Root system of the plant | 20-25 cm | 20-25 cm | 30-35 cm | 20-25 cm |
| Condition of the earth ball | Slightly compact | Slightly compact | Slightly compact | Slightly compact |
| **Disease incidence (Fungal, Bacterial, Viral, etc)** | | | | |
| Name of the disease | Free from diseases | Free from diseases | Free from collar rot disease | Free from diseases |
| **Causal organism** | - | - | - | - |
| **Detection and diagnosis** | - | - | - | - |
| **Insect pest incidence** | | | | |
| **Causal organism** | Free from pests | Free from pests | Free from pests | Free from pests |
| **Nature of damage** | - | - | - | - |
| **Detection and diagnosis** | - | - | - | - |
| **Nematode incidence** | - | - | - | - |
| **Causal organism** | - | - | - | - |
| **Nature of damage** | - | - | - | - |
| **Detection and diagnosis** | - | - | - | - |

# PART 2 : VEGETABLES

# SEED AND PLANTING MATERIAL TESTING MANUAL FOR VEGETABLES

Prepared by

Director

Indian Institute of Vegetable Research

Varanasi

**Qualitv Standards for Seed and Planting Material Testing Manual of Vegetable Crops**
**Director, IIVR, Varanasi**

### 1. Tomato

| Quality Parameters | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 70 | 70 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | none | none |
| Moisture (%) max. | | |
| Ordinary pack | 8 | 8 |
| Vapour proof pack | 6 | 6 |

### 2. Brinjal

| Quality Parameters | Standard | |
|---|---|---|
| | **Fopndation Seed** | **Certified Seed** |
| Germination (%) min. | 70 | 70 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | none | none |
| Weed seed (No./kg) max. | none | none |
| Moisture (%) max. | | |
| Ordinary pack | 8 | 8 |
| Vapour proof pack | 6 | 6 |

### 3. Chilli

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (no./kg) max. | 5 | 10 |
| Weed seed (no./kg) max. | 5 | 10 |
| Moisture (%) max. | | |
| Ordinary pack | 8 | 8 |
| Vapour proof pack | 6 | 6 |

**4. Okra**

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 65 | 65 |
| Pure seed (%) min. | 99 | 99 |
| Inert matter (%) max. | 1 | 1 |
| Other crop seed (No./kg) max. | none | 5 |
| Weed seed (No./kg) max. | none | none |
| Moisture (%) max. | | |
| Ordinary pack | 10 | 10 |
| Vapour proof pack | 8 | 8 |

**5. Cauliflower**

| Quality Parameters | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 65 | 65 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | 5 | 10 |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 5 | 5 |

**5. Cabbage**

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 70 | 70 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | 5 | 10 |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 5 | 5 |

**6. Onion**

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 70 | 70 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | 5 | 10 |
| Moisture (%) max. | | |
| Ordinary pack | 8 | 8 |
| Vapour proof pack | 6 | 6 |

**7. Vegetable pea**

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 75 | 75 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | none | 5 |
| Weed seed (No./kg) max. | none | none |
| Moisture (%) max. | | |
| Ordinary pack | 9 | 9 |
| Vapour proof pack | 8 | 8 |

**8. Cowpea**

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 75 | 75 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | 5 | 10 |
| Moisture (%) max. | | |
| Ordinary pack | 9 | 9 |
| Vapour proof pack | 8 | 8 |

## 9. French bean

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 75 | 75 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | none | none |
| Weed seed (No./kg) max. | none | 10 |
| Moisture (%) max. | | |
| Ordinary pack | 9 | 9 |
| Vapour proof pack | 7 | 7 |

## 10. Bottlegourd

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | none | none |
| Weed seed (No./kg) max. | none | none |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 6 | 6 |

## 11. Cucumber

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | None | none |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 6 | 6 |

## 12. Bittergourd

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | None | none |
| Other distinguishable varieties(No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | None | none |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 6 | 6 |

## 13. Spongegourd

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | none | none |
| Other distinguishable varieties(No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | none | none |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 6 | 6 |

## 14. Muskmelon

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation Seed** | **Certified Seed** |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | None | none |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 6 | 6 |

**15. Watermelon**

| Quality Parameter | Standard | |
|---|---|---|
| | Foundation Seed | Certified Seed |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | None | none |
| Other distinguishable varieties (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | None | none |
| , Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 6 | 6 |

**16. Pumpkin**

| Quality Parameter | Standard | |
|---|---|---|
| | Foundation Seed | Certified Seed |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other crop seed (No./kg) max. | None | none |
| Weed seed (No./kg) max. | None | none |
| Moisture (%) max. | | |
| Ordinary pack | 7 | 7 |
| Vapour proof pack | 6 | 6 |

**17. Radish**

| Quality Parameter | Standard | |
|---|---|---|
| | Foundation Seed | Certified Seed |
| Germination (%) min. | 70 | 70 |
| Pure seed (%) min. | 98 | 98 |
| Inert matter (%) max. | 2 | 2 |
| Other cro!? seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | 10 | 20 |
| Moisture (%) max. | | |
| Ordinary pack | 6 | 6 |
| Vapour proof pack | 5 | 5 |

## 18. Carrot

| Quality Parameter | Standard | |
|---|---|---|
| | Foundation Seed | Certified Seed |
| Germination (%) min. | 60 | 60 |
| Pure seed (%) min. | 95 | 95 |
| Inert matter (%) max. | 5 | 5 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | 5 | 10 |
| Moisture (%) max. | | |
| Ordinary pack | 8 | 8 |
| Vapour proof pack | 7 | 7 |

## 19. Amaranth

| Quality Parameter | Standard | |
|---|---|---|
| | Foundation Seed | Certified Seed |
| Germination (%) min. | 70 | 70 |
| Pure seed (%) min. | 95 | 95 |
| Inert matter (%) max. | 5 | 5 |
| Other crop seed (No./kg) max. | 5 | 10 |
| Weed seed (No./kg) max. | 10 | 20 |
| Moisture (%) max. | | |
| Ordinary pack | 8 | 8 |
| Vapour proog pack | 6 | 6 |

## 20. Pointed gourd

| Quality Parameter | Standard | |
|---|---|---|
| | Foundation Seed | Certified Seed |
| Approximate length of the cutting | 60 cm | 60 cm |
| Age of crop for taking cuttings | About one year | About one year |
| Age of vine to be used as cutting | 6-8 months | 6-8 months |
| Pure living planting stakes (min.) | 99.50 % (by No.) | 98.0 % (by No.) |
| Other living plants including their stem cuttings (Max.). | 0.50% | 2.00% |

**21. Garlic**

| Quality Parameter | Standard | |
|---|---|---|
| | **Foundation** | **Certified** |
| Average diameter of each bulb(min.) | 2.5cm | 2.5cm |
| Average weight of each bulb(min.) | 25g | 25g |
| No. of bulbs not conforming to varietal characteristics (max.) | 0.10% | 0.20% |
| Cut, bruised, cracked, immature or insect damaged(max. by weight) | 2.0% | 2.0% |

# PART 3 :
# SPICES

# SEED AND PLANTING MATERIAL TESTING MANUAL
# FOR
# SPICE CROPS

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## 1. BLACK PEPPER

**Name of the Crop** – Black pepper

**Botanical Name** - *Piper nigrum* L.

**Information regarding parent material**

**Age of the elite mother vine :**

- Seven years and above,
- should be a stable yielder and free from pest diseases.

**Type of planting material used for propagation-** Runner vines**.**

**Method of propagation** - Vegetatively propagated by Cutting

**Vegetative propagation**

- Age of runner vines : one year old.
- Diameter of runner vines : >0.6 cm
- Height of three node cutting(runner) : 15 cm
- Age of rooted cutting : 3 months from the date of planting in polythene bags.
- Height of the rooted cutting : 25 cm
- Number of leaves : 5 Nos.
- Diameter of the rooted cutting : > 0.8 cm
- Growth of the plant : Vigorous.
- Root system : fibrous and profusely grown.
- Condition of the earth ball : Intact and moist.
- Varietal purity should be maintained.

**Disease incidence**

i) Name of disease – *Phytophthora* infections

Causal organism – *Phytophthora capsici*

**Detection and diagnosis**

- Based on the propagule formation in water
- Based on selective isolation and culture characteristics

**ii)** Name of the disease - Anthracnose

Causal organism - *Colletotrichum gloeosporioides*

**Detection and diagnosis** - Visual.

**iii)** Name of the disease – Leaf rot

Causal organism - *Rhizoctonia solani*

**Detection and diagnosis** – Visual.

**iv)** Name of the disease - Basal wilt

Causal organism - *Sclerotium rolfsii*

**Detection and diagnosis** - Visual.

**v)** Name of the disease - Viral infections

Causal organism - *Cucumber mosaic virus* (CMV) and *Piper yellow mottle virus* (PYMV).

**Detection and diagnosis**

Direct Antibody Sandwich ELISA (DAS-ELISA) and by Polymerase chain reaction (PCR)

**Insect pest incidence**

i) Causal organism - Leaf gallthrips (*Liothrips karnyi*)

Detection and diagnosis - Visual

ii) Causal organism - Root mealybug (*Planococcus spp.*)

Detection and diagnosis -. Visual.

iii) Causal organism - Scale insects (*Lepidosaphes piperis* and *Marsipococcus marsupiale*)

Detection and diagnosis - Visual

iv) Causal organism - Top shoot borer (*Cydia hemidoxa*)

Detection and diagnosis - Visual

**Nematode incidence**

Causal organism - Root-knot nematodes (*Meloidogyne* spp.) and the burrowing nematode (*Radopholus similis*).

Nature of damage - Poor growth, foliar yellowing and inter-veinal chlorosis of leaves.

**Detection and diagnosis** - Direct examination of plant material and extraction of nematodes from roots and soil

**Nutrient deficiency Observed in Nursery**.

**Detection and Diagnosis- Magnesium**

Interveinal chlorosis of older black pepper leaves, greening of veins, necrosis and defoliation.

**Iron :** Interveinal chlorosis of younger leaves and papery whitening at severe stage.

## 2. Name of the Crop – Cardamom

**Botanical Name** - *Ellettarium cardamom*

**Information regarding parent material**

Method of propagation - Seed

**Capsule standard**

| Standards | Foundation | Certified |
|---|---|---|
| 1. Appearance | Bold and healthy | Bold and healthy |
| 2. Uniformity | >95% | >85% |
| 3. Deformed capsules | <5% | <10% |
| 4. Contamination with other varieties | <1% | <5% |
| 5. Colour of the seed | brownish black | brownish black |
| 6. Germination percent | | |
| a. Without acid treatment | 60% | 60% |
| b. With acid treatment | 80% | 70% |

**Seedling propagation**

**Seed treatment** -Acid scarification with 25% nitric acid for 10 minutes helps in early and uniform germination.

**Seedling standards**

| Standards | Foundation | Certified |
|---|---|---|
| 1. Height | 60-75 cm | 60-75 cm |
| 2. Age of the seedling | 8-10 months | 8-10 months |
| 3. No. of tillers | 3 | 3 |
| 4. Number of leaves | 5 | 5 |
| 5. Undesirable seedlings | <5 | <10 |

**Vegetative propagation**

**Standards of the planting material**

Minimum planting requirement - One grown up sucker and a growing young shoot.

Height of the main tiller - One meter

**Disease incidence**

i) Name of disease - Rhizome rot

Causal organism - *Pythium vexans* and *Rhizoctonia solani*

Nature of damage - Yellowing of leaves, leaf blades and withering of leaves.

Detection and diagnosis – Baiting the infected material.

ii) Name of disease - Viral (Katte) disease, mosaic or marble disease.

Causal organism - *Cardamom mosaic virus* (CdMV).

Nature of damage – New leaves has mosaic symptoms and green stripes.

ii) Name of disease - Cardamom vein clearing (Kokke kandu) disease

Causal organism - virus, transmitted semi persistently by *Pentalonia nigronervosa* f. *caladii*.

Detection and diagnosis for viral disease - Visual, ELISA and PCR tests.

**Insect pest incidence**

**i)** Causal organism - Shoot and capsule borer (*Conogethes punctiferalis*)

Detection and diagnosis - Visual.

ii) Causal organism - Root grubs (*Basilepta fulvicorn)*

Dection and diagnosis - Visual

iii) Causal organism - Shoot fly (*Formosina flavipes*)

Dection and Diagnosis - Visual

**Nematode incidence**

Causal organism - *Meloidogyne incognita*

Nature of damage - Poor germination, establishment , yellowing and drying of leaf tips and margins, stunting and poor growth of the plants, shedding of immature capsules in the main field, heavy galling (root-knots) and abnormal branching of roots.

Detection and diagnosis

- **Direct examination of plant material**
- Extraction of nematodes from roots and soil

## 3. Name of the Crop – Ginger

**Botanical Name - *Zingiber officinale* Rosc.**

**Information regarding parent material**

Method of propagation - Vegetative

**Preservation of seed ginger**

Seed treatment with Quinalphos 0.075% and Mancozeb 0.3% for 30 minutes, drain the solution, dry the rhizomes under shade and rhizomes are being stored in a pit.

**Standards of the planting material**

**Ginger Rhizome standards**

| Factor | Foundation | Certified |
|---|---|---|
| 1. Appearance | Healthy and Plumpy | Healthy and Plumpy |
| 2. Uniformity | 95-100% | 85-90% |
| 3. Germination | 98 | 95 |

**Disease incidence**

i) Name of the disease - Soft rot or rhizome rot disease.

Causal organism - *Pythium* species.

Nature of damage - Foul smell of the rhizomes.

Detection and diagnosis - By plating.

ii) Name of disease - Bacterial wilt

Causal organism - *Ralstonia solanacearum* Biovar-3

Detection and diagnosis - Microbiological, Serological, DAS-ELISA, NCM-ELISA and PCR tests

iii) Name of disease - Nematode incidence

Causal organism - Root knot (*Meloidogyne* spp.), burrowing (*Radopholus similis)* and lesion (*Pratylenchus* spp.)

Nature of damage - Stunting, chlorosis, poor tillering and necrosis of leaves.

Detection and diagnosis - Visual

**Insect pest incidence**

**i)** Causal organism - Rhizome scale (*Aspideilla hartii)*

Detection and Diagnosis - Visual

**4. Name of the Crop** – Turmeric

**Botanical Name** - *Curcuma longa* L.

**Information regarding parent material**

Method of propagation - Vegetative

**Preservation of seed rhizome** - Seed treatment with Quinalphos 0.075% and mancozeb 0.3% for 30 minutes, drain the solution, dry the rhizomes under shade and rhizomes are being stored in a pit.

**Standards for planting material**

**Turmeric Rhizome standards**

| Factor | Foundation | Certified |
|---|---|---|
| 1. Appearance | Healthy and Plumpy | Healthy and Plumpy |
| 2. Uniformity | 95-100% | 85-90% |
| 3. Germination | 98 | 95 |

**Disease incidence**

I Name of disease - Rhizome rot

Causal organism - *Pythium* species

Detection and diagnosis - Microbiological, Serological,DAS-ELISA, NCM-ELISA, PCR.

**Insect pest incidence**

Causal organism - Rhizomes scale (*Aspideilla hartii*)

Detection and diagnosis - Visual

## 5. Name of the Crop – Vanilla

**Botanical Name** - ***Vanilla planifolia*** **Andr**.

**Information regarding parent material**

**Method of propagation - Vegetative (Cutting)**

**Standards of planting material**

Height of cuttings - 60-120 cm in length,

Number of internodes - 15-20

Diameter - 0.8 cm.

Number of leaves - 13-15 nos

**Disease incidence**

**i) Name of disease - Stem rot**

**Causal organism** - ***Fusarium oxysporum f.sp.vanillae*** **and** ***Phytophthora meadii***

Detection and diagnosis **-Visual**

**ii) Name of disease - Root rot**

**Causal organism** – ***Phytophthora meadii*** **and** ***Sclerotium rolfsii***

**Detection and diagnosis** - Visual

**iii) Name of disease - Tip rot and die back**

**Causal organism** - ***Cucumber mosaic virus*****,** ***Cymbidium mosaic virus*****,** ***Vanilla mosaic virus.***

**Detection and diagnosis** - Visual, ELISA, Polymerase chain reaction (PCR) tests

## 6. Name of the crop - Nutmeg

**Botanical Name** - *Myristica fragrans*

**Information regarding parental material**

**Method of propagation** - **vegetative**

**Epicotyl grafting**.

**Scion Preparation**

Age of scion shoots - 3-4 month old.

Number of leaves - 2-3 leaves

Diameter of scion shoots - 0.5 cm

**Root stock preparation**

Name of the root stock - *Myristica fragrans* (seed sprout/seedlings.)

Age of the root stock - 20 days after germination at the first leaf stage.

Diameter of the rootstock - Diameter of 0.5 cm or more

Root stock propagation - Fresh Seed- undried.

**Standards of the planting material**

Height of the plant -> 15 cm

Height and condition of the union - > 15 cm, strongly united.

Scion and root diameters at the union - > 0.6 cm and above

Growth of the plant – Vigorous.

Root system of the plant - Tap root.

Condition of the earth ball - Intact and moist

**Disease incidence.**

i) Name of disease - Die back,

Causal organism - *Diplodia* sp.

Detection and Diagnosis - The disease is characterized by drying up of mature and immature branches from the tip down wards.

ii) Name of disease - Shot hole

Causal organism - *Colletotrichum gloeosporioides*.

Detection and Diagnosis - Visual

**Insect pest incidence**

**i) Causal organism** - **Black scale** (*Saissetia nigra*)

**Detection and Diagnosis** - Visual.

**Nematode incidence** **-** Nil.

# DETAILED SEED TESTING MANUAL FOR SPICES

## Introduction

India is known as land of spices. Spices are mainly used for flavouring food and in medicines. India grows over 50 different varieties of spice crops. Cultivation of spice crops in a state depends up on prevailing agro-climatic conditions. The total production of spices is around 2.7 million tonnes among which, about 0.25 million tonnes (8-10 %) is exported to more than 150 countries. During 2000-2003 spices exports quantity touched an all-time high of 2, 64, 107 tonnes. The Indian share of the world trade in spices is 45-50% by volume and 25% in value. Since Spices are of high- value and export oriented in nature, productivity of the crop is of prime importance. This can be achieved only when healthy seeds are used for propagation. Purity test, germination test, tetrazolium test, vigour test and disease testing are the important tests to judge the health of seeds, however, these tests are not applicable to spices, since they are multiplied mainly by vegetative means. The various tests to be used in the case of spices to produce quality-planting materials are dealt with here.

# I BLACK PEPPER

Black pepper (*Piper nigrum* L.) (Family: Piperaceae), the king of spices, is one of the oldest and most important spice widely used in cooking and medicine. India is a major producer, consumer and exporter of black pepper in the world. In India, Kerala and Karnataka are major producers of black pepper.

## 1.1 Propagation

Black pepper can be propagated through seeds and vegetative means. Owing to its heterozygous nature, seedlings will not breed to true to types. Hence black pepper is propagated vegetatively mainly from cuttings. Runner shoots are ideal for making cuttings.

### 1.1.1 Section of mother vines:

The runner shoots for making cuttings should be selected from uniformly established gardens free from pest and diseases. While selecting elite mother vines, following points should be kept in mind.

a) Age of the elite mother vines should 7 years and above.
b) The vine should have given a stable yield of at least 2 kg dry pepper /vine/year for 4 consecutive years and above.
c) Runner vines may be collected from a particular variety, in which purity may be maintained.

### 1.2 Test for vigour of seedlings

i) The age of the rooted cutting should be 3 months from the date of planting in the polythene bags.
ii) The rooted cutting should be of minimum height of 25 cm with 5 with vigorous growth without exhibiting any nutrient deficiency symptoms, pest and disease infection.
iii) Varietal purity should be maintained.

Black pepper rooted cuttings that fail to meet above requirements should not be considered as a quality planting material of black pepper.

## 1.3 Test for diseases

### 1.3.1 *Phytophthora* ***infections***

*Phytophthora* infections are noticed on leaves, stems and roots of cuttings in the nursery. Dark spots with fimbriate margins appear on the leaves, which spread rapidly resulting in defoliation. The infections on the stem are seen as black lesions, which result in blight. The symptoms on the roots appear as rotting of the entire root system.

#### 1.3.1.1 Detection and diagnosis

- **Based on the propagule formation in water**

The symptomatic plant parts can be immersed in sterile distilled water in a Petri-dish and incubated for 24-48h at 24-28°C under continuous light. Development of characteristic umbellate sporangial formation with long pedicels under the microscope indicates the presence of *Phytophthora.*

- **Based on selective isolation and culture characteristics**

The media for the selective isolation of *Phytophthora* from the soil is called PVPH media (Tsao, 1970). Diseased plant parts suspected to be infected with *Phytophthora* could be plated in the PVPH media after surface sterilization. Isolation of fungi from plant tissues is possible by placing small portions of infected tissue on to PVPH media in sterile petri dishes under asceptic conditions. Surface sterilization of excised tissue before plating is necessary to avoid surface contamination by saprophytic organisms that commonly grow over plant surfaces.

**Materials required**

Petri plates, razor blade, surface sterilant such as 0.1% $HgCl_2$ or 10% NaOCl., blotting paper, sterile distilled water.

**Mounting media**
**<u>Lactophenol</u>**

| | |
|---|---|
| Phenol (Pure crystal) | 20g |
| Lactic acid | 20g |
| Glycerol | 40g |
| Water | 20ml |

Heat the water using a water bath, add phenol and dissolve, and add lactic acid and glycerol

Stain
Lactophenol Cotton blue

| | |
|---|---|
| Lactophenol | 100ml |
| Cotton blue | 0.1g |

**PVPH Medium**

| | | | |
|---|---|---|---|
| Medium Ingredients : | Difco corn meal agar (CMA) | : | 17 g/L |
| | Pimaricin | : | 10 ppm (= 10 ug/ml or 10 mg/ L |
| | Vancomycin HCL | : | 200 ppm |
| | Pentachloronitro-benzene (PCNB) | : | 100 ppm |
| | Hymexazol | : | 50 ppm |

-------------------------------------------------------------------------------------------

**Procedure**

Collect leaves/stem-showing symptoms/Lesions of particular diseases. Wash the specimens thoroughly under tap water. If root tissues are used, prolonged washing may be necessary. This can be achieved by retaining root pieces in a fine sieve placed under a gentle stream of clean running tap water for 30 minutes to 2 hours.

Excise small pieces of diseased tissue from the lesion margin and surface sterilize the tissues for 1-3 minute in 10% NaOCl or 0.1% $HgCl_2$ and then wash in three changes of sterile distilled water. Blot dry on a sterile filter paper. Place on to previously poured PVPH medium in petri plate and incubate at 24-28°C. Characteristic growth appears in the medium is typical for *Phytophthora.* Further identification can be done by microscopic observation of the mycelial growth. The growth obtained in the medium can be incubated under continuous light for 24-48 hrs. The *Phytophthora* causing foot rot namely *P. capsici* can be identified by the microscopic staining procedure.

**Procedure for staining**

Take clean microscopic glass slides without oil trace and place a drop of lactophenol/water/lactophenol cotton blue. Carefully withdraw a small pinch of fungal

growth aseptically from the culture tube/plate with an inoculation needle (Platinum loop holder) by holding the tube against a spirit lamp. Place the fungal pinch on the slide in the drop of lactophenol and gently separate the hyphae using a sterile needle. Place a coverglass over the mount. Remove excess of stain/ mountant using filter paper and observe under the microscope. Permanent preparation may be made in lactophenol/ lacto phenol cotton blue and seal the sides of the cover glass using nail polish.

**Detection from soil through baiting**

The presence of *Phytophthora* in the soil can be detected and quantified using the baiting technique. *Phytophthora capsici* can be isolated using leaves of *Albizzia* sp. as bait.

**Procedure**

- Collect soil along with roots from the rhizosphere of black pepper plant affected by foot rot.
- Place around 50 g of the soil in a beaker.
- Add 10ml of distilled water and mix the contents of the beaker thoroughly using a glass rod.
- Put 10-20 leaf lets of Albizzia sp. Cover the beaker with thin polythene sheet to retain humidity and incubate under laboratory conditions of 20-24°C.
- Daily observe for infection of leaves (usually infection starts with in 24h).
- Remove the infected leaves and place it on a glass slide and observe for *Phytophthora* growth under the microscope.

<u>Observation</u>

Positive baiting showes the presence of *Phytophthora* sporangia all along the periphery of the leaves. The infected bits can be placed under the microscope to see the characteristic umbellate sporangial ontogeny typical of *P. capsici* all over the periphery of the leaves.

**Identification of *P. capsici***

The *P. capsici* isolate is characterized by the umbellate branching of sporangia with long pedicel having a length of 15-200 µm.

### 1.3.2 Stunted disease (Viral disease)

Vein clearing, mosaic, yellow specks, mottling and small leaf are the most obvious symptoms for identifying viral infections in the nursery. As viruses are systematic in nature, primary spread occurs through plating material since black pepper is vegetatively propagated. When infected plants are used as source of planting material, the cuttings will also be infected. Hence selection of virus-free healthy mother plants is very important. Secondary spread of the disease occurs through insects such as aphids and mealy bugs. Because of closed placing of seedlings in the nursery, chances of spread through these insects are more.

#### 1.3.2.1 Detection and diagnosis

Two viruses namely, *Cucumber mosaic virus* (CMV) and *Piper yellow mottle virus* (PYMV) are associated with the disease. Use of infected planting material is the main source for virus spread. Hence it is essential to detect planting material for the presence of viruses before they are planted. Direct antibody sandwich **e**nzyme linked immunosorbent assay (DAS-ELISA) and Polymerase chain reaction (PCR) are the two methods used for the detection of these viruses.

**Direct Antibody Sandwich ELISA (DAS-ELISA)**

In DAS-ELISA, Immunoglobulin G (IgG) extracted from virus specific antiserum are used for coating the solid surface to trap the antigen, and the same IgG is labeled with an enzyme are employed for detection. The antigen gets sandwiched between IgG's and thus is referred to as the double antibody sandwich (DAS) form of ELISA. The reaction is visualized by addition of substrate of the enzyme used for labeling leading to the development of colour. The intensity of the colour is proportional to the concentration of antigen present.

Requirements

**Materials**

Microtitre (ELISA) plate: Polystyrene make of any reliable brand may be used
Micropipette and tips: Use adjustable volume micropipette.

Multichannel pipette (adjustable100 to 300 μl) (Optional): Multichannel pipettes which can hold four, eight or 12 microtips thus permitting dispensing of volumes simultaneously into several wells can be used to add antigen, antiserum and conjugates.

Incubator, beakers, magnetic stirrer, pH meter

ELISA plate reader

Virus infected and healthy tissue.

**Reagents**

IgG (against virus of interest)

IgG-alkaline phosphatase conjugate

**Coating buffer (Carbonate buffer):** Dissolve 1.59 g $Na_2CO_3$, 2.93 g $NaHCO_3$, and 0.20 g $NaN_3$ in one litre of distilled water.

Phosphate buffered saline (PBS)

**PBS-T:** Add 0.05% Tween 20 in 1X PBS

**Antigen extraction /antibody buffer:** Add 2% polyvinyl pyrrolidone (PVP, MW 40,000) and 0.2% albumin in PBS-T.

**Substrate buffer**: Dissolve 97 ml diethanolamine in 800 ml distilled water, adjust pH to 9.8 with concentrated Hcl, make up the volume using distilled to 1000 ml.

**Para nitrophenyl phosphate (PNPP):** Commercial preparation available either in powder or tablet forms may be used. Protect from light while storing.

Protocol

1. Add 200 μl of IgG diluted in coating buffer to each well of microtitre plate and incubate at 37°C for 2 h.
2. Well contents are discarded and washed with PBS-T (flooding with three changes of PBS-T for three min each time). After final washing, plates are shaken dry over paper towel.
3. Test samples are extracted in antigen extraction buffer in 1:10 (w/v) using pestle and mortar. Extracts of healthy plants and the extraction buffer are to be used as negative control, whereas, extracts from known infected plant should be used as positive control. 200 μl aliquots of test sample are added and plates incubated overnight at 4°C.

4. The plates are washed as mentioned in (2) and diluted enzyme conjugate (200 µl per well) in antibody buffer is added and incubated at 37$^o$C for 2 h.
5. After washing as described in (2), 200 µl of substrate (p-nitro phenyl phosphate 0.6 mg/ml of substrate buffer) is added to each well and incubated at room temperature to develop color.
6. Results are recorded by measuring the absorbance at 405 nm in an ELISA reader after 30, 60 and 120 minutes after substrate addition.
7. Stop the reaction if required by adding 100 µl of 3 M NaOH solution to each well.

**Note:**

1. Use appropriate dilutions of antigens, antisera and conjugates. Usually, antigen is used at 1:5 or 1:10 dilution while conjugates are diluted according to their titre value.
2. In each plate include appropriate controls, such as buffer, negative (healthy) and positive (infected).
3. Include at least two or three replications for each of the samples.
4. For better results use, freshly prepared substrate buffer.
5. PNPP is photodegradable. Hence avoid exposure to light. Cover the beaker with aluminium foil to avoid direct contact with sunlight Add as quickly as possible once PNPP solution is prepared.
6. An O D value more than twice that of healthy should be considered as positive for the presence of virus.

### 1.4.2 PCR for detection of *Piper yellow mottle virus*

As nucleotide sequence of the portion of PYMV genome is available, PCR can be used to detect this virus in planting materials. The important steps in the PCR include: (i) extraction of total DNA from virus infected plant or from purified virus preparations; (ii) synthesis of two (forward and reverse) virus specific primers; (iii) set up the PCR reaction in a vial by adding extracted nucleic acid, primers, nucleotides, magnesium chloride and *Taq* DNA polymerase. Use nucleic acid extracted from a known infected and healthy plants to serve as positive and negative controls respectively; (iii) keep the vials in the PCR machine and start the run as per the pre-decided program and (iv)

identify positive reactions by running contents of PCR on the agarose gel. The presence of bands at the expected position indicate that sample under test is positive.

**Requirements**

**Materials**

Thermal cycler
Microcentrifuge
Micropipette and tips
Eppendorf tubes
Thin walled PCR tubes
Ice flaking machine and ice
Agarose
Microwave
Gel apparatus with power pack
Transilluminator

**Reagents**

Template DNA
10 x PCR buffer
$MgCl_2$
dNTP mix
Primers
*Taq* DNA polymerase
TAE buffer
Ethidium bromide
Gel loading dye

**Protocol**
1. In a sterile 0.2 ml thin walled PCR tube, add the following

| | |
|---|---|
| 10 x PCR buffer | 5.0 µl |
| 10 mM dNTP mix | 1.0 µl |
| 100 ng/µl Forward Primer | 0.5 µl |
| 100 ng/µl ReversePrimer | 0.5µl |
| Taq DNA polymerase (3Units/µl) | 0.5 µl |
| Sterile water | 32.5 -37.5 µl |
| Template DNA | 5-10 µl |
| Total volume | 50µl |

Also, set up similar reaction using template DNA from a known template (positive control) and another reaction without any template (negative control).

2. Amplify the nucleic acid using the denaturation, annealing and extension times, and temperatures required for each of the steps. Times and temperatures needed to be set up depend on the primer annealing temperature and the length of fragment being amplified. Extension should be carried out for 1 min for every 1000 base pair of length of fragment being amplified. Denaturing and extension are usually carried out at 94°C and 72°C respectively. Temperature for annealing of primers should be either Tm or Tm-2°C of the primers.

3. Run about 10-15 µl of the reaction mixture in 1% agarose gel for 1 h. through electrophoresis. Include DNA markers of appropriate size.

4. Visualize the DNA by placing the gel on a transilluminator.

**Results**

A successful amplification reaction should yield a readily visible DNA fragment of expected size. The gel containing samples of positive controls and template DNA under test should contain a prominent band of DNA of appropriate molecular weight. This band should be absent from the lanes containing samples of negative controls.

**Reverse transcription polymerase chain reaction (RT-PCR) for the detection of** *Cucumber* ***mosaic virus***

RT-PCR is a method to amplify complementory DNA (cDNA) copies of RNA. In this, the first step is the enzymatic conversion of RNA to a single stranded cDNA template. An oligo deoxynucleotide primer is hybridized to the mRNA and is then extended by an RNA-dependent DNA polymerase (Reverse transcriptase) to create cDNA copy that can be amplified by PCR. Either RNA isolated from purified viral preparations or total RNA isolated from infected plant or dsRNA isolated from infected plant can be used as template to initiate cDNA synthesis. The primer for first strand cDNA synthesis can be specifically designed to hybridize to a particular target. Amplification of the desired portion of cDNA can be achieved in PCRs primed by sense and antisense oligonucleotide primers corresponding to specific sequences in particular cDNAs. Positive and negative controls should always be included when setting up RT-PCRs.

**Requirements**

**Materials**

Micropipettes, tips, eppendorf tubes, gloves
Thin walled tubes for amplification
Water bath
PCR work station
Thermal cycler
Agarose
Gel apparatus with power pack
Microcentrifuge

**Reagents**

10X PCR amplificatin buffer
10 mM dNTP mix0.1M Dithreitol
$MgCl_2$
RNase inhibitor
Reverse transcriptase
Taq DNA polymerase
RNase free water
Agarose gel
RNA template (either isolated from purified virus or total RNA isolated from infected plants).
Oligonucleotide primers (both sense and antisense).

## Protocol

Assemble RT-PCR reaction components on wet ice and prepare amplification mix into PCR tube in order given below.

| | |
|---|---|
| 10X PCR buffer | 10.0 µl |
| 0.1 M DTT | 10.0 µl |
| 25 mM $MgCl_2$ | 5.0 µl |
| 10 mM dNTP mix | 1.0 µl |
| Forward primer | 1.0 µl |
| Reverse primer | 1.0 µl |
| rRNasin (32 units/µl) | 0.5 µl |
| AMV RT (20 units/µl) | 1.0 µl |
| Taq DNA polymerase | 1.0 µl |
| Template RNA* | 40.0 µl |
| Water | to make 100.0µl |

*Should be denatured by heating at 75°C for 10 minutes and snap cooling on ice for at least 3 min before adding into PCR mix.

Mix reaction components assembly by inverting and place the tube on a thermal cycler and proceed with the thermal cycle profile chosen for the reaction.

Temperature programme for RT-PCR reaction (25-40 cycles)

| Step | Temp (°C) | Duration |
|---|---|---|
| Reverse transcription | 42 | 45 min |
| Denaturation | 90-94 | 30 –60 sec |
| Annealing* | 37-55 | 30- 60 sec |
| Extension$ | 72 | 1- 3 min |
| Final extension | 72 | 10 min |

* depend on the Tm of primers.
$ Provide one minute for every 1000 bp to be amplified.

Run about 10-20 µl of reaction on 1% agarose gel and visualize the DNA.

**Results:**
A successful amplification reaction should yield a readily visible DNA fragment of expected size. The gel containing samples of positive controls and template DNA under test should contain a prominent band of DNA of appropriate molecular weight. This band should be absent from the lanes containing samples of negative controls.

## 1.4 Nematode infestations

Root-knot nematodes (*Meloidogyne* spp.) and the burrowing nematode (*Radopholus similis*) are the two important nematode species infesting rooted cutting in the nursery. The damage caused to roots by nematode infestations result in poor growth, foliar yellowing and some times inter-veinal chlorosis of leaves. The establishment of nematode infected cuttings will be poor when planted in the field and such cuttings develop slow decline symptoms at a later date.

### 1.4.1 Direct examination of plant material

#### 1.4.1.1 Detection and diagnosis

Select a few rooted cuttings at random from the nursery. Remove the soil and wash the roots in water to observe the root system carefully. Observe the roots keenly for the presence of lesions or galls or rotting. Selected roots with lesions or galls or rotting may be teased apart using sharp needles or forceps under a stereomicroscope at magnification

from 15 to 50 X to confirm the presence of nematodes. Migratory endoparasites like Radopholus emerge in a few minutes and can be found moving about. Presence of galls is a clear indication of root-knot nematode infection. They may be seen attached or embedded in the root tissue.

1.4.1.2 Extraction *of nematodes from roots*

Roots of black pepper rooted cuttings are gently washed free from soil or debris and are sliced into small bits of 2-3 cm size. They are first cleared in diluted sodium hypochlorite bleach (5.25% NaOCl) for about 4 min. Remove all traces of the bleach by thoroughly rinsing in water. Take about 1-2 g roots in a 200 ml glass vial and cover it with acid fuchsin solution (875 ml of lactic acid, 63 ml of glycerol, 62 ml of water, 0.1 g of acid fuchsin). Boil the solution for 30 s in a microwave oven or on a hot plate in a ventilated area. Allow the root tissue to cool and wash off the excess stain in running tap water. Macerate the stained roots in an electric mixer to extract the nematodes from the plant tissue. Drawing aliquots from this suspension and counting under a stereomicroscope can quantify the nematode infection in roots.

1.4.1.3 Extraction of nematodes from soil

Soil collected from polythene bags containing rooted cuttings is mixed thoroughly and about 100 ml is taken in a plastic basin. Add about 3-4 l of water and stir to free nematodes. The suspension is first poured through a 2 mm aperture sieve into another basin avoiding the sediment. The suspension in the second basin is then poured through another sieve of 125 µm aperture and the process is repeated using 38 or 25 µm aperture sieve. The residues from the above two sieves are pooled and rinsed repeatedly to get a clean suspension that is collected in a 100 ml beaker. The contents are allowed to settle for 1-2 h and the supernatant liquid is carefully decanted. The remaining material is observed under a stereoscope for the presence of nematodes.

1.4.1.4 Storage of samples

**Samples are collected in clean polythene bags, sealed and stored under warm (16-18°C) conditions till they are processed. If samples have to be stored for longer**

**duration, the roots may be fixed in hot (60-70°C) formal acetic acid (Formaldehyde – Acetic acid, 4:1)**

## 1.5 Insect pests

### 1.5.1 Leaf gall thrips (*Liothrips karnyi*)

1.5.1.1 Symptoms and Diagnosis

Infestation by leaf gall thrips (Liothrips karnyi) is more serious at higher altitudes. The feeding activity of thrips on tender leaves causes the leaf margins to curl downwards and inwards resulting in the formation of marginal leaf galls and crinkling and malformation of leaves. The symptoms of pest infestation include presence of marginal leaf galls, crinkled and malformed leaves. The adults are black and measure 2.5 - 3.0 mm in length. The larvae and pupae are creamy white.

### 1.5.2 Root mealybug (Planococcus spp.)

#### 1.5.2.1 Symptoms and Diagnosis

Root mealybugs infest roots and bases of nursery plants especially at higher altitudes. The symptoms of pest infestation include yellowing, wilting and stunting of plants. The base of stems and main roots are generally infested with mealybug colonies. Many of the plants infested with root mealybugs are often infested by *Phytophthora capsici*, the fungal pathogen and plant parasitic nematodes such as *Meloidogyne incognita* and *Radopholus similis*. In such plants the symptoms are more severe and they succumb to the pest and fungal attack within a shorter period. Root mealybugs are small, oval, soft-bodied insects measuring about 1.5 mm x 1.0 mm in size and the body is covered with white waxy filaments. Mealy bugs are tended by ants and the occurrence of ant colonies at the base of the plants is an indication of the pest infestation.

### 1.5.3 Scale insects (*Lepidosaphes piperis* and *Marsipococcus marsupiale*)

1.5.3.1 Symptoms and Diagnosis

Among the various scale insects recorded on black pepper, *L. piperis* and *M. marsupiale* sometimes infest older cuttings in nurseries especially at higher altitudes. The pest

infestation results in yellowing, wilting and drying of leaves and is more severe during the post monsoon and summer periods. *L. piperis* appear as encrustations on stems and leaves and the females are elongated (about 1mm length) and dark brown. *M. marsupiale* mainly infests leaves and the females are oval to triangular (about 1.5 mm) and yellow brown and also result in sooty mould formation.

### 1.5.4 Top shoot borer (*Cydia hemidoxa*)

1.5.4.1 Symptoms and Diagnosis

The top shoot borer is occasionally seen in nurseries where the bamboo method is adopted for multiplication of plants. The larvae bore into tender terminal resulting in blackening and decaying of tender terminal shoots of mother plants. The affected shoots are hollow when split open. The adult is a tiny moth with a wingspan of 10-15 mm with crimson and yellow fore wings and grey hind wings. Fully-grown larvae are grayish green and measure 12-15 mm in length.

## 1.6 Quality requirement under the seed act in terms of tolerance limit for pests/ diseases

| Sl. No | Pest/diseases | Tolerance limit (%) |
|---|---|---|
| a | *Phytophthora* foot rot diseases | 1.0 |
| b | *Radopholus similis* | 1.0 |
| c | *Meloidogyne incognita* | 5.0 |
| d | Stunted disease | 1.0 |
| e | Scale insect | 1.0 |
| f | Root mealybug | 1.0 |
| g | Leaf gall thrips | 5.0 |
| h | Top shoot borer | 5.0 |

# II.CARDAMOM

Cardamom (*Elettaria cardamomum* Maton) is propularly known as queen of spices and belongs to family Zingiberace. It is grown in Western Ghats of Kerala, Karnataka and Tamil Nadu. It is a cross pollinated crop and propagated by seeds and suckers. Due to heterozygous nature of seed propagated material, clonal propagation and planting of high yielding clones is presently followed on large scale in Kerala and to a lesser extent in Tamil Nadu and Karnataka where, seed propagation is still in practice for large scale planting.

## 2.1 Propagation

### 2.1.1 Seedlings propagation

For raising the seedlings high yielding selections/consistent high yielders have to be selected. The selected plants should have > 2 panicles per yielding tillers. The panicles should posses > 15 racemes with bold capsules having >15 seeds. Seed capsules have to be harvested from 2 $^{nd}$ years crop from 3$^{rd}$ or 4$^{th}$ round of harvest (September). After dehusking, seeds are soaked in water, mucilage is removed by repeated washing and floats are discarded. Acid scarification with 25% nitric acid for 10 min helps in early and uniform germination. Early germinated seedlings of 4-5 leaf stages are transplanted to polybags / raised beds provided with 50 % shade. 8-10 months old seedling having minimum 3 tillers are to be selected for field planting.

### 2.1.2 Rapid clonal propagation

High yielding varieties/selections are generally multiplied in isolated clonal nurseries. Virus free high yielding plants are selected and subcloned for further multiplication. For rapid multiplication following timely agro techniques has to be followed

1. High yielding plants free from pest and diseases, with characters like bold capsules and retentivity of green colour are to be selected from plantations and part of the clump has to be uprooted for clonal multiplication leaving the mother clump in its original place to induce subsequent suckers for further use.
2. The minimum planting unit consists of one grown up sucker (rhizome) and a growing young shoot.

3. Trenches having width and depth of 45 cm and convenient length have to be opened filled with jungle soil, compost and topsoil.
4. The rhizomes (planting unit) are placed at a spacing of 1.8 m x 0.6 m in trenches, thus accommodating 9259 plants per hectare of clonal nursery area.
5. Pandal protection, regular watering (once in a week during November to May) and chemical manure @ 48:48:96 g. NPK per plant in two splits have to be applied.
6. On an average 32 - 42 suckers will be produced after 12 months of planting per one planting unit. Taking the barely minimum of 50% of these suckers/clump one can get 16-21 planting units (one grown up sucker along with a growing young shoot *i.e.* sucker) from one mother-planting unit after 12 months.
7. In an area of 1-hectare clonal nursery 1,48,144 to 1,94,439 planting units can be produced after 12 months.
8. Clones thus produced should be free from virus, rhizome rot and root knot nematodes.

## 2.2 Test for Identification of diseases

### 2.2.1 Rhizome rot

This disease caused by *Pythium vexans* and *Rhizoctonia solani* takes a heavy toll in the old nurseries. Excesive soil moisture and lack of proper drainage in the nursery are the predisposing factors for infection by *P. vexans* whereas damping off caused by Rhizoctonia appears when warm temperature prevails. When this disease occurs in mature plants it is known as rhizome rot (clump rot). The initial disease symptoms are noticed on leaves, which show slight paleness and yellowing of leaves at the top. Gradually yellowing spreads into leaf blades and leaf sheaths followed by withering of plans. In primary nursery, infected seedlings collapse at collar region and die in patches. In grown up seedlings the infection starts from collar and spreads into rhizome, which first becomes discoloured, and decay. The pseudostems and rhizomes of grown up plants when infected become soft, ultimately resulting in the death of the clump. The Rhizoctonia infection is indicated in the form of brownish discoloration in the collar, whereas pinkish discoloration and soft decay is the symptom of *Pythium* infection.

#### 2.2.1.1 Detection and diagnosis

Presence of *Pythium* or *Fusarium* can be detected by baiting the infected material in water. Characteristic growth of *Pythium* or *Fusarium* formed in water indicates its association with the disease. The subglobose or pyriform, intercalary or terminal 15-20µ long and av 17.9µ broad sporangia can identify *P.vexans* with bell shaped antheridia. *P.vexans* can be identified by the subglobose or pyriform, intercalary or terminal 15-20µ long and av 17.9µ broad sporangia with bell shaped antheridia. The monoclinous bell shaped antheridia easily distingush *P. vexans* from other species with spherical and aplerotic oospores. *Fusarium oxysporum* can be identified by the delicate mycelium with white, peach or purple tinge. Micro conidia borne on simple phialids arising laterally on the hyphae or from short sparsely branched conidiophores. Micro conidia generally abundant. Macro conidia are thin walled , 3-5 septate fusoid –subulate and pointed at both ends. *Rhizoctonia solani* can be distinguished by the brownish fast growing mycelium with long cells, septa of branch set off from main hyphae and form brown sclerotia.

## 2.3 Plant parasitic nematodes

Root-knot nematode *Meloidogyne incognita* is a serious problem in cardamom. They are present both in the nurseries and plantations of the entire cardamom growing tracts of South India. They feed on the roots; make the plants weak and vulnerable to diseases. In their presence the nursery diseases like rhizome rot and damping off due to *Rhizoctonia solani* gets aggravated.

### 2.3.1 Nature of damage

Poor germination of seeds in the primary nurseries, poor establishment after transplanting to secondary nurseries or main fields, yellowing and drying of leaf tips and margins, stunting and poor growth of the plants, shedding of immature capsules in the main field, heavy galling (root-knots) and abnormal branching of roots. (Galling is prominent in seedlings while in the mature plants the galls are small in size with abnormal branching of roots, which are devoid of rootlets).

#### 2.3.1.2 Detection and diagnosis

2.3.1.2.1 Direct examination of plant material

Remove a few seedlings with their root system intact from nursery beds where symptoms like stunted growth, yellowing etc. are observed. The soil is separated and the roots are washed gently in water. Observe the root system carefully for the presence of galls or any malformation. Dip the roots in Phloxine B (0.15 g/l of water) stain for 15-20 min, rinse and examine in water. The root-knot nematode egg masses will be stained red. Or else the selected roots with galls may be teased apart using sharp needles or forceps under a stereomicroscope at magnification from 15 to 50 X to confirm the presence of nematodes.

2.3.1.2.2 Extraction of nematodes from roots and soil

For extraction of nematodes from soil and roots the methods described under black pepper (A2 and A3) may be followed.

## 2.4 Viral diseases

### 2.4.1 Katte disease (Mosaic or marble disease)

The first visible symptom appears on the youngest leaf of the affected tiller as spindle shaped slender chlorotic flecks measuring 2-5 mm in length. Later these flecks develop into pale green discontinuous stripes. The stripes run parallel to the vein from the midrib to leaf margin. All the subsequently emerging new leaves show characteristic mosaic symptoms with chlorotic and green stripes. The disease is caused by *Cardamom mosaic virus* (CdMV), spreads through aphid vector *Pentalonia nigronervosa* f. *caladii* and also by the use of infected planting material. The virus is non-persistent and stylet borne. This disease is not transmited through seed or soil; however the disease adversely affects seed germination.

### 2.4.2 Cardamom vein clearing (Kokke kandu) disease

This disease reported so far only from India is of relatively recent origin has become a threat to cardamom cultivation in few endemic pockets of Karnataka. Because of its characteristic symptom, it is locally referred to as *kokke kandu*, The first visible

symptoms of the disease include continuous or discontinuous vein clearing. Later rosetting, loosening of leaf sheath and shredding of leaves were seen. New leaves get entangled in the older leaves and form hook like tiller hence the name *kokke kandu*. The disease is caused by a virus and transmitted semipersistently by *Pentalonia nigronervosa* f. *caladii*. This disease is not transmited through seed or soil; however the disease adversely affects the seed germination.

#### 2.4.2.1 Detection and diagnosis for viral diseases

Examine plants for symptoms. As symptoms cannot be the sole criteria for identifying disease free nature of the planting material, it is necessary to perform ELISA or PCR tests. The details of methodology for the same are explained in pages in black pepper.

## 2.5 Insect pests

### 2.5.1 Shoot and capsule borer (*Conogethes punctiferalis*)

#### 2.5.1.1 Symptoms and diagnosis

The shoot and capsule borer is a seroius pest in the nursery and the early stages of the larva bores in to un opened leaf buds and the later stage larvae bore the pseudostem and feed the central core resulting in drying of the terminal leaf. The dried terminal leaf resulting in dead heart is a characteristic symptom of the post infestation. The conspicuous oozing out of frass from the bore-hole on the pseudostem is an indication of the presence of larva inside the plant. The adult is a medium-sized, orange yellow moth with black spots and with a wingspan of 20-25 mm. The fully-grown larvae are dull brown with sparsc hairs and are 15-25 mm in length.

### 2.5.2 Root grubs (*Basilepta fulvicorn)*

#### 2.5.2.1 Symptoms and Diagnosis

Root grubs cause serious damage to roots of suckers in the nursery resulting in yellowing and drying of leaves, stunting of plants and rotting of roots. The infested plants can be easily pulled off from the soil. Adult beetles are shiny, metallic blue, blusih green, greening brown or brown and measure 4-6 nm length. The mature grubs are short, stout, C shaped, pale white and 1 cm in length.

### 2.5.3 Shoot fly (*Formosina flavipes*)

#### 2.5.3.1 Symptoms and Diagnosis

The larvae of shoot fly feeds on the growing shoot of suckers in the resulting in yellowing and drying of terminal shoot and dead heart symptoms. The grubs are minute and are seen at the base of the growing central shoot near the rhizome.

**6. Quality requirement under the seed act in terms of tolerance limit for pests/ diseases**

| Sl. No | Pest/diseases | Tolerance limit |
|---|---|---|
| a | Rhizome rot | 1.0 |
| b | Nematodes (root knot) | 5.0 |
| c | Katte | 1.0 |
| d | Kokke kandu | 1.0 |
| e | Root grub | 1.0 |
| f | Shoot borer | 5.0 |
| g | Shoot fly | 1.0 |

# III GINGER

Ginger (*Zingiber officinale* Rosc.), (Family: Zingiberaceae), a native of tropical South East Asia, is mainly cultivated in tropical and subtropical countries. The underground rhizome is used as spice. Though ginger is cultivated in many states, Kerala, Meghalaya and Arunachal Pradesh are leading producers of ginger in the country.

## 3.1 Propagation

### 3.1.1 Selection of mother rhizomes

Seeds should be collected from healthy, disease and pest free beds, which should be marked in the field when the crop is 6-8 months old and still green. Beds having minor disease incidence should be rejected for collecting the seed rhizome.

### 3.1.2 Preservation of seed ginger

After harvest, big plumpy rhizomes are washed thoroughly in water to remove the soil and dried in shade for a day. The seed rhizomes are treated with a solution containing Quinalphos 0.075% and mancozeb 0.3% for 30 minutes, drain the solution and dry the rhizomes under shade. Dried rhizomes are put in a pit of convenient size (1x1 m) and covered with a plank fitted with 2-3 holes for aeration. The seed rate ranges from1000-1500 Kg/ha. Small rhizome pieces of 2.5-5 cm length weighing 20-25 g each with at least two buds are used for sowing.

## 3.2 Tests for Diseases

### 3.2.1 Soft rot or rhizome rot disease

Soft rot is the most destructive disease of ginger that results in total loss of affected clumps. The disease is rhizome and soil-borne, and is caused by *Pythium* spp. The affected rhizome gives a characteristic foul smell.

#### 3.2.1.1 Detection of soft rot causing fungi, *Pythium* sp from rhizomes

*Pythium* can be detected in rhizome by plating ginger rhizome samples directly onto the medium in the petriplate

**Composition of Potato Dextrose Agar medium ($gL^{-1}$)**

| | |
|---|---|
| Potato | :200 |
| Dextrose | :20 |
| Agar | :18 |
| Water | :1000 ml |

Potatoes were scrubbed to clean and cut in to small cubes. Two hundred g weighed, rinsed rapidly in tap water and boiled in 1000 ml of water until soft. After filtered through muslin cloth added agar and boiled to dissolve agar. Dextrose was added to this solution stirred to dissolve and made up to 1000 ml. Dispensed 100 ml aliquots in 250ml conical flasks and autoclaved at $120^{0}$C for 20 minutes.

For detection of *Pythium* the method based on direct isolation on selective medium is available. Ten ml of the following $P_{10}$VP solution was added to 100 ml of molten Potato Dextrose Agar medium and poured it into the sterile Petri plates.

**Preparation of $P_{10}$VP stock solution**

**$P_{10}$ VP Medium**

| | | | |
|---|---|---|---|
| Medium Ingredients : | Difco corn meal Agar (CMA) | : | 17 g/L |
| | Pimaricin | : | 10 ppm (= 10 ug/ml or 10 mg/ L |
| | Vancomycin HCL | : | 200 ppm |
| | Pentachloronitro-benzene (PCNB) | : | 100 ppm |

After making the selective medium, the suspected rhizome sample is directly plated onto the medium and incubated for 2-3 days for colony development.

### 3.3.1 Bacterial wilt

Bacterial wilt caused by *Ralstonia solanacearum* Biovar-3 is also a soil and seed borne disease that results in rotting of affected rhizomes.

#### 3.3.1.1 Detection of bacterial wilt pathogen *Ralstonia solanacearum*

**Microbiological methods**

The suspected samples can be directly plated onto a medium specific for *R. solanacearum*. Extract from ginger rhizomes is directly plated onto the following specific medium for isolation of *R.solanacearum* and its detection. The colonies developed after 36 hours with fluidal nature, pink center with irregular margins confirmed to be *Ralstonia solanacearum.*

**Composition of specific medium ($gL^{-1}$)**

| | |
|---|---|
| Potatoes | 200.0 |
| Bactopeptone | 10.0 |
| Dextrose | 2.5 |
| Cassamino acid | 1.0 |
| Distilled water | 1.0 L |
| Agar | 18 |

This basal medium was autoclaved and then cooled to 50°C and the following filter sterilized solutions of antibiotics and vitamin C were added aseptically per liter of medium.

| | |
|---|---|
| 1% Polymyxin B sulphate (100 mg/ml) | 10 ml |
| 1% Cyclohexamide (100 mg/l) | 10 ml |
| 1% Bacitracin (25mg /l) | 2.5 ml |
| 0.1% Penicillin – G (0.5 mg/l) | 500 µl |
| 1% Chloramphenicol (5 g/l) | 500 µl |
| 1% Crystal violet (5 mg/l) | 500 µl |
| 1% 2,3,5 -Triphenyl tetrazolium chloride (TZC 50 mg) | 5.0 ml |

(2.5ml of vitamin C solution prepared by dissolving a tablet containing 500mg vitamin C in 20 ml of distilled water (62.5mg/l)

**Serological methods.**

Serological method is available for detection of bacterial wilt pathogen in ginger. Various techniques including serological methods have been developed for the detection of *R. solanacearum* in soil, plant materials and irrigation water (Seal, 1997)

Latently infected rhizomes have been implicated in the transmission and spread of bacterial wilt of crop plants especially in ginger. The sensitivity, short time, high speed and low cost are the main advantages of serological methods. The DAS-ELISA methodology is furnished below

**Enrichment of *R. solanacearum* extracted from ginger rhizomes**

**Composition of Enrichment Broth**

Same as *R. solanacearum* specific medium except that it does not contain the solidifying agent, agar.

Enrichment broth (4.5 ml) was dispensed in 30 ml sterile vials and 1ml of $10^{-5}$ dilution of the rhizome wash and vascular extract was transferred to separate vials. This was incubated for 48 hours at $30^0$C with constant agitation (180 rpm) in an incubator shaker.

*Coating the microtitration plate with R solanacearum specific rabbit immunoglobulins (IgG)*

To 12.5 ml of coating buffer, 100µl of *R solanacearum* – specific rabbit IgG is added.

**Composition of coating buffer (g)**

| | | |
|---|---|---|
| $Na_2CO_3$ | : | 0.159 |
| $NaHCO_3$ | : | 0.293 |
| $NaN_3$ | : | 0.020 |
| Distilled water | : | 100 ml |

125 µl of the coating solution was added to each well of the microtitration plate and the plate was covered with a piece of parafilm to prevent evaporation and it was incubated at 37°C for 4 hours.

**Application of the samples to the wells of the microtitration plate.**

The excess coating solution was discarded from the microtitration plate and the plate was washed with washing buffer.

Composition of washing buffer

| | | |
|---|---|---|
| Tween 20 | : | 250 µl |
| PBS buffer | : | 500 ml with sodium azide |

**Composition of PBS buffer ($gL^{-1}$)**

| | | |
|---|---|---|
| NaCl | : | 8.0 |
| $KH_2PO_4$ | : | 0.2 |
| KCl | : | 0.2 |
| $Na_2HPO_4$ | : | 1.15 |
| $NaN_3$ | : | 0.2 |

The washing is done in microplate washer (Immunowash, BIORAD, Model 1575) as follows: The wells are filled with washing buffer and it is left for 3-4 minutes in the carrier of immunowash. The plate is then emptied and refilled with washing buffer and the process is continued for two more times. After last washing the plate is put upside down on a paper towel and tapped it several times to remove all buffer.

With a micropipette, a 125 µl sample of one of the enriched sample is transfixed to each of two wells of the microtitration plate. This is repeated for all samples. The positive and negative control is also added to the plate.

Note: The positive controls are boiled suspensions of R *solanacearum* at $10^8$, $10^7$ $10^6$and $10^5$ bacteria/ml and the negative control is an enriched extract of R *solanacearum* - free soil supplied along with the kit. The plate is sealed with parafilm and incubated at 4°C in a standard refrigerator for 18 hours (overnight).

**Addition of enzyme-labeled *R solanacearum* -specific rabbit immunoglobulins (conjugated IgG)**

After 18 hours, the plate is washed as previously described until plate is completely colorless. After discarding the last washing buffer, 125µl of conjugate solution is added to each well of the plate. The plate is sealed as before and incubated it at 37°C for 4 hours.

**Conjugate solution**

| | |
|---|---|
| R *solanacearum* specific rabbit IgG conjugated to alkaline phosphatase | 100 µl |
| Conjugate | 12. 5ml |

**Conjugate buffer**

| | |
|---|---|
| PVP-4000 | 0.250 g |

Non-fat powdered milk 0.025 g

### Development of colour

The conjugate solution from the plate is discarded and the unbound conjugated IgG is removed by washing the plate with washing buffer as previously described. After discarding the last washing buffer, 125 µl of the color development solution is added to each well of the plate.

### Preparation of colour development solution

One tablet (5 mg) of substrate P-np (P-nitrophenylphosphate) is dissolved in 12.5 ml of substrate buffer and pH is adjusted to 9.8 by adding 1N NaOH.

**Composition of substrate buffer (pH 9.8)**

| | | |
|---|---|---|
| Diethanolamine | : | 10.9 ml |
| HCl (37%) | : | 1.50 ml |
| Distilled water | : | 6.00 ml |

After adding solution to all the wells as quickly as possible, the plate is left for 60 minutes at room temperature (20-25$^{o}$C) for the colour to develop. The colour formation of the positive samples is observed and measured using spectrophotometer (µQuant, Microplate Reader, Bio-Tek, USA) at 405 nm wavelengths.

Note: The samples are considered positive if the absorbance is three times as much as the average of the enriched soil solution free of *R. solanacearum* used as negative control.

**NCM-ELISA methodology:** This is one the qualitative method for detection of the pathogen in suspected sample with out the aid of Plate Reader.

Rhizomes harvested for seed purpose can be tested for *R. solanacearum* in post enrichment NCM-ELISA. Enrichment is performed by incubating the rhizome extract as described above in selective medium at 30°C with constant agitation (180 rpm). An 8 x 12 cm of 0.45 mm pore size nitro cellulose membrane (NCM) (Biorad) is immersed for 5 minutes in 30 ml Tris Buffered Saline buffer (TBS) (0.02 mol l -1 Tris-HCl [pH 7.5], 0.05 mol l -

1 NaCl, 0.01% NaN 3) for NCM-ELISA. Twenty µl of the enriched suspension are put on the membrane manually, transferred to a dry filter paper and air-dried for at least 60 minutes. The NCM dotted with the samples is incubated for 1 h in 30 ml of the blocking solution (2% non-fat powdered milk in TBS buffer) in a Petri dish (15 cm diameter), with gentle rotary agitation (50 rpm) and the membrane is incubated for 2 h with gentle agitation in 30 ml of the antibody solution (100 ml of *R. solanacearum*-specific antiserum diluted 1:1000 is added to another 30 ml of the same blocking solution). The membrane is washed to remove the unbound *R. solanacearum* antibodies with 30 ml

T-TBS (TBS with 0.05% Tween-20) three times for 3 minutes each with constant agitation at 100 rpm. Then, the membrane is incubated for 1 h with gentle agitation with 30 ml of the conjugated solution, containing goat-antirabbit antibodies conjugated to alkaline phosphatase (Biorad), diluted 1: 4000 in 30 ml of the blocking solution. The membrane is rinsed three times for 3 minutes each with T-TBS, with constant agitation (100 rpm) to remove the unbound conjugate. During the last washing, the colour development (substrate) solution is prepared by adding drop by drop while agitating, first 100 ml of NBT (p-nitro blue tetrazolium) solution and then 100 ml of BCIP (p-toluidine salt of 5 bromo, 4-chloro, 3-indolyl phosphate) solution in a dark flask containing 25 ml of substrate buffer (0.1 mol l -1 Tris base, 0.1 mol l -1 NaCl, 0.005 mol l -1 MgCl 2, 6H 2 O, pH 9.6). The membrane is then incubated with 25 ml of the substrate solution with gentle agitation for 5 to 30 minutes. The reaction is stopped by discarding the substrate solution and by rinsing the membrane thoroughly with tap water. The membrane is then placed on filter paper sheets to dry. Development of colour can be compared to positive control strips for *R. solanacearum*.

It is recommended to test at least 100 samples in a lot of one ton of ginger. If 10% of the samples test positive for the pathogen, then the particular lot can be rejected.

**Detection of *Ralstonia solanacearum* through PCR**

This is one of the most confirmatory assays for detecting the presence of the pathogen in rhizome (or even in soil). DNA from rhizomes of ginger can be isolated using CTAB method. However it is recommended to use DNA isolation kits PCR amplification for

detection of *R. solanacearum* in soil is performed using the DNA isolated from rhizome. Reaction volume (25µl) contained PCR buffer (Mo Bio, USA), $MgCl_2$: 1.5 mM (Promega Corporation, USA), dNTP mix: 0.05mM (Mo Bio, USA), Polymerase enzyme: 0.5u, Template DNA: 100 ng, BSA: 10µg, Primers: 20 pmoles each (Rs Primer1: 5'-gTC gCC gTC AAC TCA CTT TCC-3'; Rs Primer 2: 5'-gTC gCC gTC AgC AAT gCg gAA TCg-3').

PCR is performed in thermal cycler at the following PCR conditions as shown below and the final PCR products are resolved in 1.5 or 2.0 % agarose in 1x Tris Acetate EDTA buffer at 4°C for 6 hours at 4V/cm. The gel is stained with Ethidium bromide and photographed on an UV transilluminator and the results are documented in Alpha imager 2002 for analysis.

Thermal cycling conditions for ***Ralstonia solanacearum*** specific primer

| *Ralstonia solanacearum* specific primer | |
|---|---|
| Initial denaturation at 94°C for 3 min, annealed at 53°C for 1 min and extended at 72°C for 1.5 min | |
| 94 °C for 15 s<br>60 °C for 15 s<br>72°C for 15 s | X 30 cycles |
| Final extension 72°C for 5 min | |

Primer sequence mentioned above is known to amplify 281bp sequence in the genomic DNA of *Ralstonia solanacearum*, which has been exploited in the detection assay for *R. solanacearum* using PCR.

### 3.4.1 Tests for nematode pests

Root knot (*Meloidogyne* spp.), burrowing (*Radopholus similis)* and lesion (*Pratylenchus* spp.) nematodes are important nematode pests of ginger. Stunting, chlorosis, poor tillering and necrosis of leaves are the common aerial symptoms. Characteristic root galls and lesions that lead to rotting are generally seen in roots. The infested rhizomes have brown, water soaked areas in the outer tissues. Nematodes infestation aggravates rhizome rot disease.

#### 3.4.1.1 Detection and diagnosis

Direct examination of plant material

Collect seed rhizomes at random and examine for symptoms like dry rot. Using a sharp knife cut the rhizomes across and observe for brown patches or necrotic areas. Such pieces are teased apart in water under a stereoscope. Active nematodes can be seen wriggling out of the tissue.

## 3.3 Insect pests

### 3.3.1 Rhizome scale (*Aspideilla hartii)*

**Symptoms and Diagnosis**

The rhizome scale infests rhizomes in storage feeding on sap resulting in shriveling and desiccation of rhizomes. Adult (female) scales are circular (about 1mm diameter) and light brown to grey and appear as encrustations on the rhizomes.

**Quality requirement under the seed act in terms of tolerance limit for pests/ iseases**

| Sl. No | Pest/diseases | Tolerance limit (%) |
|---|---|---|
| a | Rhizome rot | 1.0 |
| b | Bacterial wilt | 1.0 |
| c | Rhizome scale | 1.0 |
| d | Nematodes | 5.0 |

## IV. TURMERIC

Turmeric (*Curcuma longa* L.) (Family: Zingiberaceae) is cultivated mainly for its culinary and medicinal values and the dried rhizomes or underground stems yield the spice of commerce. India is a leading producer of turmeric in the world. Andhra Pradesh, Tamil Nadu and Orissa are the major turmeric production states in India.

### 4.1 Propagation

#### 4.1.1 Preservation of seed rhizome

Depending upon the variety, the crop becomes ready for harvest in 7-9 months after planting during January-March. Bold and healthy rhizomes for seed purpose are stored by heaping in well-ventilated rooms and covered with turmeric leaves**.**The seed rhizomes can also be stored in pits with sawdust, sand, leaves of *Glycosmis pentaphylla,* etc. The pits are to be covered with wooden planks with one or two openings for aeration.To protect the rhizomes from scale and fungi , rrhizomes are dipped in quinal phos 0.075% solution and Mancozeb 0.3 % for 30 minutes and dried before storage.

### 4.2 Test for diseases

#### 4.2.1 Rhizome rot

The disease is caused by *Pythium spp.* Infected rhizomes appear soft resulting in rot.

##### 4.2.1.1 Detection and diagnosis

Methodology used for the detection of rhizome rot of ginger is employed (refer pages19, 20).

### 4.3 Test for nematode pests

Root knot nematodes (*Meloidogyne* spp.) and burrowing nematodes cause damage to turmeric. Root lesion nematodes (*Pratylenchus* spp.) are of common occurrence in Andhra Pradesh.

4.3.1 Direct examination of plant material

Collect seed rhizomes at random and examine for symptoms like dry rot. Using a sharp knife cut the rhizomes across and observes for brown patches or necrotic areas. Such pieces are teased apart in water under a stereoscope. Active nematodes can be seen wriggling out of the tissue.

## 4.4 Test for insect pests

### 4.4.1 Rhizome scale (*Aspideilla hartii)*

#### 4.4.1.1 Symptoms and Diagnosis

The rhizome scale infests rhizomes in storage feeding on sap resulting in shriveling and desiccation of rhizomes. Adult (female) scales are circular (about 1mm diameter) and light brown to grey and appear as encrustations on the rhizomes.

**Quality requirement under the seed act in terms of tolerance limit for pests/ diseases**

| Sl. No | Pest/diseases | Tolerance limit (%) |
|---|---|---|
| a | Rhizome rot | 1.0 |
| b | Bacterial wilt | 1.0 |
| c | Rhizome scale | 1.0 |
| d | Nematodes | 1.0 |

## V. VANILLA

Vanilla (*Vanilla planifolia* Andr.), (Family: Orchidaceae), a native of Mexico, is cultivated to a limited extent in Kerala, Karnataka and Tamil Nadu. The crop has gained considerable importance in recent years due to increased demand for natural vanillin that is extracted from its pods.

### 5.1 Propagation

#### 5.1.1 Selection of mother vines

Vigorously growing healthy disease and pest free vines are selected from yielding plants for the collection of cuttings. Vines of current years growth with dormant auxiliary vegetative buds can be selected for taking stem cuttings. Longer cuttings having 60-120 cm in length, with 15-20 internodes 13-15 leaves are ideal for planting. Over matured part of vanilla plant and flowered one may not be used for the selection of cuttings.

### 5.2 Test for Diseases

5.2.1 Stem Rot

5.2.1.1 Detection and diagnosis

**The disease usually appears during the post monsoon periods of November-February months, when the temperature is very low and humidity relatively very high. The disease appears in the form of water soaked lesions extending to both sides of the stem forming a yellow coloration. When the basal or middle portions of the vines decay and shrivel, the remaining distal portions of the vines show wilting symptoms. Vine rot and drying are generally observed at the basal portions above the ground level. The infected areas showed decaying symptoms and the remaining portions wilt off as they have insufficient number of aerial roots. The causal organisms were identified as *Fusarium oxysporum f.sp.vanillae* & *Phytophthora meadii.* The method of identification of the organisms is the same as described for capsule rot & rhizome rot of cardamom**

5.2.2 Root Rot

5.2.2.1 Detection and diagnosis

Initially the disease appears in the form of browning and death of both underground aerial roots. Aerial roots may be produced in large numbers but most of them die before entering the soil and results in flaccidity and shriveling of the stem and finally the vine droops. During the periods of drought the vines become less capable of withstanding the infection due to inadequate uptake of water and minerals, excess sunlight and excess bearing of fruits due to over pollination. The causal organisms of the disease include *Phytophthora meadii* and *Sclerotium rolfsii Sclerotium rolfsii* can be identified by the formation of pure white thick mycelial mat and white to brownish mustard like sclerotia over the infected areas. Culturing of the infected portions in Potato-Dextrose agar will also show the pure white mycelial mat with in 48hrs and white to brown sclerotial formation in seven days.

### 5.2.3 Tip rot and die back

#### 5.2.3.1 Detection and diagnosis

Visible symptoms of rotting develop on the petiole and lower portions of the youngest leaf. Within a few days the rotting extends to the whole funnel like leaf and the shoot apex become soft brown and droop down. During rainy season, the rotting and dieback extends downwards until the next node. The causal organism(s) of the disease include *Phytophthora meadii* or *Fusarium oxysporum.*

**The method of identification of the organisms is the same as described in rhizome rot of cardamom. (Isolation procedures were the same as described for foot rot disease of black pepper. For fungi other than *Phytophthora* or *Pythium*, Potato Dextrose agar can be used instead of PVPH or $P_{10}$VP medium.)**

### 5.2.4 Test for viral infection

#### 5.2.4.1 Mosaic and necrosis diseases

##### 5.2.4.1.1 Detection and diagnosis

Various kinds of mosaic such as mild mottle, mild mosaic and mild chlorotic streak (could be seen when the leaf is held against light) are observed. In a few cases, such mosaics are also associated with leaf distortion with wavy margin. The size of the leaves also gets reduced and in advanced stages, leaves become brittle and show severe crinkling. The association of *Cucumber mosaic virus*, *Cymbidium mosaic virus*, *Vanilla mosaic virus* have been reported with the disease. The necrosis disease is characterized by the appearance of brown necrotic patches on the stem region with shriveled appearance. The affected stem shows distinct necrotic lesions of varying length (few mm to several cm). This disease is different from the fungal induced stem rot. Stem necrosis can be distinguished from stem rot caused by fungi by the following:

- Stem rot affected region will be totally blighted and very soft which can be easily felt by touching the affected region, while stem necrosis (caused by viruses) affected region when touched appear very dry and hard and gives cracking sound when attempted to break open. Fungal diseases are commonly seen during the wet monsoon period whereas stem necrosis is seen all though the year.
- A close look at the stem rot affected region show a white cottony growth on the upper surface of the affected region while no such growth is seen with stem necrosis affected region.

- In case of stem rot the portion above the lesion often wilts yellowing of leaves whereas in stem necrosis no wilting would be seen.

In a few cases, necrosis is also seen on the leaves at the lower surface in the form of scab. This often gives the appearance of sun scorch. The disease initially starts as a necrotic spot on the stem and slowly gets enlarged and encircles the stem. In an affected plant, necrosis may be seen only at one or few regions on the stem. Rest of the stem region looks apparently healthy without any visual symptoms. A few of the necrosis affected plants also show mosaic symptoms on leaves.

The major means of spread of the virus is though the use of infected stem cuttings. Insects may also play an important role in the transmission and spread of the disease in nature.

The presence of viruses in the planting material may be confirmed through sensitive laboratory tests such as enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). The detailed procedure of both these techniques have been given in pages 5-8.

**5.3 Quality requirement under the seed act in terms of tolerance limit for pests/diseases**

| Sl. No | Pest/diseases | Tolerance limit (%) |
|---|---|---|
| a | Fungal diseases (Stem/root rot) | 1.0 |
| b | Viral diseases (Mosaic and necrosis) | 1.0 |

## SEED TESTING FOR SEED SPICES

The seed spices have emerged as one of the important group of spices crops in India. All most all states in India grow one or more seed spices. But major growing area lies in arid to semi-arid regions mainly in the states of Rajasthan and Gujarat and some extent in Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh and Chattisgarh. About 50 varieties of different seed spice crops have been released but sufficient quantity of quality seeds are not available to the farmers. The quality seed of seed spices is a pre-requisite for successful raising of these crops. The quality seed production requires information on seed certification standards particularly the field and seed standards. The minimum seed certification standards and necessary guidelines on the quality seed production of seed spices are warranted by the seed producers and related agencies time and again.

### 6.1 Seed quality parameters:

There are four parameters considered important in quality seed production such as physical purity, germinability, health and genetic purity.

The seed spices crops *viz.,* coriander, cumin, fennel ajowain and dill belong to family Apiaceae and are mostly cross pollinated in nature. The cross pollination occurs mostly through insects (entomophilous). The coriander and cumin are considered as often cross pollinated, whereas fennel, ajowain and dill are cross pollinated crops. The fenugreek belonging to Leguminosae family is self pollinated in nature. The isolation distance to be kept between the fields of two varieties to produce foundation and certified seed, respectively are given in Table 1.

#### 6.1.1 Isolation distance for seed production in seed spice crops

| Name of crop | Distance in meters | |
|---|---|---|
| | Foundation seed | Certified seed |
| Fenugreek (highly self pollinated) | 50 | 25 |
| Coriander (often cross-pollinated) | 800 | 400 |
| Cumin (often cross pollinated) | 800 | 400 |

| Fennel (cross pollinated) | 1000 | 800 |
|---|---|---|
| Ajowain (cross pollinated) | 1000 | 800 |
| Dill (cross pollinated) | 1000 | 800 |

The specific requirement for seed crop of seed spices showing maximum permitted limits for off types, objectionable weeds, and designated diseases are given in Table 2.

### 6.1.2 Purity Standards of Seed Spices

| Name of crop | Maximum permitted limits (%) | | | | | |
|---|---|---|---|---|---|---|
| | Foundation seed | | | Certified seed | | |
| | Off type (un wanted seed) | Objectionable weed | Diseased plants | Off type/ Un-wanted seed | Objectionable weed | Diseased plants |
| Coriander | 0.10 | - | 0.10 | 0.50 | - | 0.50 |
| Cumin | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| Fennel | 0.10 | - | 0.10 | 0.20 | - | 0.50 |
| Fenugreek | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| Ajowain | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| Dill | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |

The objectionable weeds and designated diseases as reported for various seed spices crops are given here.

### 6.1.3 Objectionable weeds in seed spices:

Cumin : *Plantago pumila* (jiri)
Fenugreek : *Melilotus* (Senji)
Ajowain : *Trachyspermum roxumburghai*

### 6.1.4 Designated seed borne and other diseases in seed spices:

Coriander **:** Fusarium wilt (*Fusarium oxysporum* f.sp.*coriandrii*), stem gall (*Protomyces Macrosporus),* powdery mildew *(Erisiphae polygoni)*

Cumin **:** Fusarium wilt (*Fusarium oxysporum* f.sp. *cumini),* Cumin blight (*Alterneria Burnsii),* powdery mildew (*Erisiphae polygoni)*

Fennel : Alterneria blight (*Alterneria tenuis*) and *Ramularia* blight (*Ramularia foeniculi*)

Fenugreek : Root rot (*Rhizoctonia solani*), downy mildew (*Peronospora trigonellae*), leaf spot (*Cercospora traversiana*), powdery mildew (*Erisiphae polygoni*)

Ajowain : Collart and root rot (*Sclerotium rolfsii*), blight (*Alternaria* spp.)

Dill : Root rot F(*Fusarium oxysporum*), powdery mildew(*Erisiphae polygoni*)

### 6.1.5 Seed standards in seed spices:

The seed spice crops meeting field standards for certification should be harvested, threshed and processed as per guidelines issued by the certification agency. Soon after the completion of seed processing the composite sample is taken for analysis of the seed standards such as percentage of pure seed, inert matter, other crop seed, total weed seed, germination and moisture. In order to ensure quality seed production, it is necessary to strictly follow the seed standards. The prescribed permittable seed standard limits of seed spices crops is given in Table 3. On receipt of Seed analysis Report and the result of grow-out-test, the packing, tagging, sealing follows the issuance of certificate.

**6.1.6 Seed standards for Seed Spices**

| Name of crop | Seed standard (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pure seed (minimum) | | Inert matter (maximum) | | Seed of other crop (maximum) | | Total weed Seed (maximum) | | Germination (%) | | Moisture (%) | |
| | FS | CS | FS | CS | FS | CS | FS | CS | FS | CS | FS | CS |
| Coriander | 98 | 97 | 2 | 3 | 0.10 | 0.20 | 0.10 | 0.20 | 65 | 65 | 10 | 10 |
| Cumin | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 65 | 65 | 8 | 8 |
| Fennel | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 70 | 70 | 8 | 8 |
| Fenugreek | 98 | 98 | 2 | 2 | 0.10 | 0.20 | 0.10 | 0.20 | 70 | 70 | 8 | 8 |
| Ajowain | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 65 | 65 | 8 | 8 |
| Dill | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 70 | 70 | 8 | 8 |

**FS** - Foundation Seed; **CS -** Certified Seed

Note:

1. Insect damaged seeds should not be more than 0.5% in each sample.
2. The moisture % for vapour proof container should be 2% less than the prescribed limit.

The seed quality control is not an easy task. It requires lot of planning and efforts on the part of government. The production and marketing agencies are also equally responsible to maintain and preserve the seed quality to the highest possible standard.

**The detailed procedure / methodology for seed testing is given in Annexure – I.**

## 6.2 SUBMITTING SEED SAMPLES FOR TESTING

### 6.2.1 Timing

Timing of seed stocks one to two months prior to planting should reflect seed quality following storage and handling.

### 6.2.2 Obtain representative sample:

Take several grab samples or use a grain spear to collect a representative sample of the grain. Avoid the temptation to take the whole sample from the soil or augerhopper. These samples can give a biased indication of seed viability. Contact a seed-testing laboratory that can recommend a sampling procedure for your particular situation.

### 6.2.3 SEED SAMPLING

The first step in seed testing is sampling. ***Seed lot***: It is a uniformly blended quantity of seed either in bag or in bulk.

| Seed size | Maximum quantity per lot |
|---|---|
| Larger than wheat and paddy | 20,000 kg |
| Smaller than wheat and paddy | 10,000 kg |
| Maize | 40,000 kg |

### 6.2.4 Details are to be attached with the samples.

- Name and address
- Kind of seed and variety
- Year of harvest
- Type of test required (e.g. germination, purity or tetrazolium), and then forward the sample to be tested to the seed laboratory of your choice.

### 6.2.5 Sampling intensity

**For seed lots in bags (or container of similar capacity that are uniform in size)**

| | |
|---|---|
| Up to 5 containers | Sample each container but never < 5 primary samples |
| 6-30 containers | Sample atleast one in every 3 containers but never < than 5 primary samples |
| 31-400 containers | Sample atleast one in every5 containers but never < than 10 primary samples |
| 401 or more containers | Sample atleast one in every 7 containers but never < than 80 primary samples |

When the seed is in small containers such as tins, cartons or packets a 100 kg weight is taken as the basic unit and small containers are combined to form sampling units not exceeding these weight e.g. 20 containers of 5 kg each. For sampling purpose each unit is regarded as one container.

**For seeds in bulk**

| | |
|---|---|
| Up to 500 kgs | At least 5 primary samples |
| 501 to 3000 | Primary samples for each 300 kg but not less than 5 P.S |
| 3001-20,000 | Primary samples for each 500kg but not less than 10 P.s |
| 20,001 and above | Primary samples for each 700 kg but not less than 40. |

## 6.2.6 METHODS OF SAMPLING

### 6.2.6.1 Hand sampling

This is followed for sampling the non-free flowing seeds or chaffy and fuzzy seeds such as cotton, tomato, grass seeds etc., In this method it is very difficult to take samples from the deeper layers of bag. To over come this, bags are emptied completely or partly and then seed samples are taken. While removing the samples from the containers, care should be taken to close the fingers tightly so that no seeds escape.

#### 6.2.6.2 Sampling with triers

By using appropriate triers samples can be taken from bags or from bulk.

Bin samplers: Used for drawing samples from the lots stored in the bins.

Nobbe trier: This is suitable for sampling seeds in bag, not in bulk.

Sleeve type triers or stick triers: It is the most commonly used trier for sampling. There are two types *viz., w*ith compartments and without compartments. This trier is used for drawing seed samples from the seed lots packed in bags or in containers.

### 6.2.7 Weight of submitted sample

The minimum weight for submitted samples for various tests are as follows:

**Moisture test**

100 grams for those species that have to be ground and 50 grams for all other species.

### 6.2.8 For verification of species and cultivar

| Crop | Lab only (g) | Field plot & lab (g) |
|---|---|---|
| Peas, beans, maize, soybean and crop seeds of similar size | 1000 | 2000 |
| Barley, oats, wheat and crop seeds of similar size | 500 | 1000 |
| Beetroot and seeds of similar size | 200 | 500 |
| All other genera | 100 | 200 |

### 6.2.9 For other tests like purity and count of other species

| Crop | Size of seed lot (kg) | Size of submitted sample (g) | Size of working sample for purity (g) | Size of working sample for count of other species (g) |
|---|---|---|---|---|
| Coriander<br>Fennel | 10,000<br>10,000 | 400<br>180 | 40<br>18 | 400<br>180 |
| Fenugreek | 10,000 | 450 | 45 | 450 |

The samples taken may be packed in bags, sealed and marked for identification. For moisture testing the samples should be packed separately in moisture proof polythene bag and kept in the container along with submitted samples.

### 6.2.10 Information to accompany the sample

Date, Kind, Variety, Class of seed, Lot No., and Quantity of seed in lot (kg), Sender's name and address. Test(s) required: (1) Purity (2) Germination and (3) Moisture.

### 6.2.11 Types of sample used in seed testing lab

Service sample : Sample received from the farmers.

Certified sample : Sample received from certification agencies or officers.

Official sample : Sample received from the seed inspectors.

## 6.3 MIXING AND DIVIDING OF SEEDS

### 6.3.1 Mechanical dividing

The reduction of sample size is carried out by the mechanical dividers suitable for all seeds except for chaffy and fuzzy seeds.

Soil divider: It is suitable for large seeds and chaffy seeds.

### 6.3.2 Random cups method

This method is suitable for seeds requiring working sample upto 10 grams provided that they are not extremely chaffy and do not bounce or roll (e.g) *Brassica spp*. Six to eight small cups are placed at random on a tray. After a preliminary mixing, the

seed is poured uniformly over the tray. The seeds that fall into the cup is taken as the working sample.

### 6.3.3 Spoon method

This is suitable for samples of single small seeded species. Sufficient portions of seed are taken to estimate a working sample of approximately but not less than the required size.

### 6.3.4 Hand halving method

This method is restricted to the chaffy seeds. The halved portions are arranged in rows and alternate portions are combined and retained. The process is repeated until the sample of required weight is obtained.

## 6.4 PHYSICAL PURITY ANALYSIS

6.4.1 Purity test

A purity test is the analysis of a representative sample of the seed tonnage for contaminants, such as weed seeds, other crop seeds, broken seed, soil, insects and any other contaminants. Any prohibited matter (Seeds and live insects) found during the purity analysis are identified and entered on the report. Seed must not be sold until prohibited material is removed from the seed lot. The purity test does not cover the genetic purity of the sample.

*Pure **seed:*** The seeds of kind / species stated by the sender. It includes all botanical varieties of that kind / species. Immature, undersized, shrivelled, diseased or germinated seeds are also pure seeds. It also includes broken seeds, if the size is > 1/2 of the original size except in leguminosae and cruciferae, where the seed coats entirely removed are regarded as inert matter.

***Other crop seed*:** It refers to the seeds of crops other than the kind being examined.

*Weed seed***:** It includes seeds of those species normally recognized as weeds or specified under seed act as a noxious weed.

*Inert matter***:** It includes seed like structures, stem pieces, leaves, sand particles, stone particles, empty glumes, lemmas, paleas and chaff. Awn stalks longer than florets, spikelets are to be removed and treated as inert matter.

**Method:**

The Working sample:

The purity analysis is done on the working sample of prescribed weight drawn from submitted samples. The analysis may be made on one working sample of the prescribed weight, or on two sub-samples of at least half this weight, each independently drawn.

Weighing The working sample

The number of decimal places to which the working sample and the components of the working sample should be weighed as below:

| Weight of the working sample in gram | Number of decimal places required | Example |
|---|---|---|
| <1 | 4 | 0.7534 |
| 1-9.999 | 3 | 7.534 |
| 10-99.99 | 2 | 75.34 |
| 100-999.9 | 1 | 753.4 |
| 1000 or more | 0 | 753.4 |

**Physical separation:**

The working sample after weighing is separated into its components *viz.*, pure seed, other crop seed, weed seed and inert matter.

**Method of purity separation:**

Place the sample on the purity work board after sieving / blowing operations and separate into other crop seeds, weed seeds and inert matter. After separation identify each kind of weed seeds, other crop seeds as to genus and species. The names and number of each are recorded.The type of inert matter present should also be noted.

**Calculation:**

All the four components must be weighed to the required number of decimal places. The percentage of the components are determined as follows:

$$\% \text{ of component} = \frac{\text{Weight of individual component}}{\text{Total weight of all components}} \times 100$$

If there is a gain or loss between the weight of the original samples and the sum of all the four components is in excess of one percent, another analysis should be made.

Duplicate tests

Analysis result near the borderline in relation to the seed standards, one more test is done and the average is reported. However, if a duplicate analysis is made of two half samples, or whole samples, the difference between the two must not exceed the permissible tolerance.

Determination of inseparable other crop seeds and objectionable weed seeds (by Number /Kg)

Whole submitted sample is used and the number per kg may be calculated and reported even if the working sample is less than a Kg.

Determination of other distinguishable varieties **(ODV)**

Ten times, the size of working sample is used. It is determined based on the morphological characters of the seeds. The authentic samples should be available for comparison. The number of ODV should be calculated and reported as No./kg of seeds.

Calculation of results:

The % by weight of each of the component should be calculated to one decimal place.

Reporting results:

The results of each component are given in one decimal place and the total of all components must be 100. Components of < 0.05% shall be reported as Trace. If the result for a components is nil, this must be shown as '-0.0-' in the appropriate space.

**6.4.2 Equipment used for purity analysis**

Seed Blower

There are 2 plastic columns one for larger seeds and the other for smaller seeds. The plastic column is provided with a semi-circular outlet where the terminal velocity of

wind can be adjusted. A time clock is also provided for the automatic running of the bowler. The inert matter is separated by stratification using the terminal velocity of air.

Purity work board

This is used for effective separation of different components. At the centre of the board, there is an illumination by which the emptiness of the seed is easily identified.

## 6.4 GERMINATION TEST

Germination tests shall be conducted with the pure seed fraction. A minimum of 400 seeds are required in four replicates of 100 seeds each or 8 replicates of 50 seeds each or 16 replicates of 25 seeds each depending on the size of the seeds and size of containers of substrate. The test is conducted under favourable conditions of moisture, temperature, suitable substratum and light if necessary. No pretreatment to the seed is given except for those recommended by ISTA.

Medium

The medium serves as moisture reservoir and provides a surface or medium for which the seeds can germinate and the seedlings grow. The commonly used substrata are sand, paper and soil.

**Sand**

Size of sand particle: Sand particles should not be too large or too small. The sand particles should pass through 0.80 mm sieve and retained by 0.05 mm sieve.

Toxicity: Sand should not have any toxic material of any pathogen. If any pathogen is found, then the sand should be sterilized in an autoclave.

Germination tray: When we use the sand, germination trays are used to carry out the test. The normal size of the tray is 22.5 x 22.5 x 4 cm. The tray may be either zinc or stainless steel.

**Method of seed treatment**

Seeds in sand (s): Seeds are planted in a uniform layer of moist sand and then covered to a depth of 1 cm to 2 cm with sand.

Top of sand (TS): Seeds are pressed into the surface of the sand.

Spacing: We must give equal spacing on all sides to facilitate normal growth of the seedling and to avoid entangling of seed and spread of disease.Spacing should be 1-5 times the width or diameter of the seed.

Water: The amount of water to be added to the sand will depend on size of the seed. For cereals, except maize, the sand can be moistened to 50% of its water holding capacity. For large seeded legumes & maize sand is moistened to 60% WHC.

**PAPER**

Most widely used paper substrates are filter paper, blotter or towel (kraft paper). It should have capillary movement of water, at vertical direction (30 mm rise / min.) .It should be free from toxic substances and free from fungi or bacteria. It should hold sufficient moisture during the period of test. The texture should be such that the roots of germinating seedlings will grow on and not into the paper.

Methods

Top of paper (TP): Seeds are placed on one or more layers of moist filter paper or blotter paper in petridishes. The petridishes are covered with lid and placed inside the germination cabinet. This is suitable for those seeds, which require light.

Between paper (BP): The seeds are germinated between two layers of paper.

Roll towel method: The seeds are placed between two layers of paper and rolled in towels. The rolled towels are placed in the germinator in an upright position.

Inclined plate method: Germination on glass plate with germination paper and kept at an angle of $45^o$.

**Soil**

Should be non-caking, free from any large particles. It must be free from weed seeds, bacteria, fungi, nematode or toxic substances. Soil is not recommended for reuse.

Temperature: Required temperature is maintained (most seeds germinate between 20-$30^oC$)

Light: Light should be provided for seeds requiring light for germination (e.g.) lettuce and tobacco.

### 6.5.2 Germination appratus

Germination cabinet / Germinator: This is closed chamber where in temperature and relative humidity are controlled. We can maintain the required temperature.

Room germinator: It works with same principles as that of germinator. This is modified chamber of larger one and the worker can enter into it and evaluate the seedlings. Provisions are made to maintain the temperature and relative humidity. This is used widely in practice.

Counting board: This is used for accurate counting and spacing of seeds. This consists of 2 plates. The basal one is stationary and top one is movable. Both top and basal plates are having uniform number of holes *viz.*, 50/100, when the plates are in different position. After taking the sample, the top plate is pulled in such a way that the holes are in one line so that the fixed numbers of seeds fall on the substratum.

Vacuum counter: Consists of head, pipe and wall. There are plates of 50 or 100 holes which can be fitted to the head. When vacuum is created the plate absorbs seeds and once the vacuum is released the seeds fall on the substrate.

Impression board: Made of plastic / wood with 50 or 100 holes per pins. Here the knobs are arranged in equal length and space. By giving impression on the sand it makes uniform depth and spacing for seed.

### 6.5.3 GERMINATION REQUIREMENTS FOR DIFFERENT CROPS

| Crop | Substratum | Temp. ($^0$ C) | Light | First count days | Final Count days | Recommendation for breaking dormancy |
|---|---|---|---|---|---|---|
| Coriander (*Corian*dum *sativum* I) | TP,BP | 20-30, 20 | L | 5 | 10 | 7 |
| Fennel (*Foeniculam vulgar*e Mill) | TP,BP,TS | 20-30 | L | 5 | 14 | Light |
| Fenugreek (*Trigonella foenumgraecum* L) | TP,BP | 20-30, 20 | - | 4 | 14 | Light |
| Cumin (*Cuminum cyminum*) | TP,BP | 20-30 | - | 5 | 7 | - |

TP- TOP of paper
BP- Between paper
TS- Top of Sand

## 6.6 Seedling evaluation

ISTA classified the seedlings into different categories based on the development of essential structures.

### 6.6.1 Categories of seedlings

**Normal seedlings**

Seedlings which show the capacity for continued development into normal plant when grown in favourable conditions of soil, water, temperature and light.

**Characters of normal seedlings**

a. A, well-developed root system with primary root except in certain species of graminae which normally producing seminal root or secondary root.
b. A, well-developed shoot axis consists of elongated hypocotyl in seedlings of epigeal germination.
c. A, well-developed epicotyl in seedlings of hypogeal germination.
d. One cotyledon in monocotyledons and two in dicotyledons
e. A, well-developed coleoptile in graminae containing a green leaf.
f. A, well-developed plumule in dicotyledons.
g. Seedlings with following slight defects are also taken as normal seedlings. Primary root with limited damage but well developed secondary roots in leguminaosae (Phaseolus, Pisum) graminae (maize), cucurbitaceae (cucumis) and malvaceae (cotton).
h. Seedlings with limited damage or decay to essential structures but no damage to conducting tissue.
i. Seedlings, which are decayed by pathogen but it, is clearly evident that the parent seed is not the source of infection.

**Abnormal seedlings**

Seedlings which do not show the capacity for continued development into normal plant when grown in favourable conditions of soil, water, temperature and light.

**Type of abnormal seedlings:**

Damaged seedlings: Seedlings with any one of the essential structures are missing or badly damaged so that the balanced growth is not expected. Seedlings with no cotyledons, with splits, cracks and lesions on essential structures and without primary root.

Deformed seedlings: Weak or unbalanced development of essential structures such as spirally twisted or stunted plumule or hypocotyl or epicotyl, swollen shoot, stunted roots etc.,

Decayed seedlings: Seedlings with any one of the essential structures showing diseases or decayed symptoms as a result of primary infection from the seed which prevents the development of the seedlings.

**Hard seeds**

Seeds which do not absorb moisture till the end of the test period and remain hard (e.g.) seeds of leguminosae and malvaceae.

**Fresh ungerminated seeds**

Seeds which are neither hard nor have germinated but remain firm and apparently viable at the end of the test period.

**Dead seeds.**

Seeds at the end of the test period are neither hard nor fresh or have produced any part of the seedlings. Often dead seeds collapse and a milky paste come out, when pressed at the end of the test.

**6.6.2 Retesting**

If the results of a test are considered unsatisfactory it shall not be reported and a second test shall be made by the same method or by alternative method under the following circumstances.

a. Replicates performance is out of tolerance

b. Results being inaccurate due to wrong evaluating of seedlings or counting or errors in test conditions.
c. Dormancy persistence or phytotoxicity or spread of fungi or bacteria. The average of the two tests shall be reported.

**6.6.3 Use of tolerances**

The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances. To decide if two test results of the same sample are compatible again the tolerance table is used.

**6.6.4 maximum tolerated ranges between replicates**

| Average percentage germination | | Maximum range | Average percentage germination | | Maximum range |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 1 | 2 | 3 |
| 99 | 2 | 5 | 87 to 88 | 13 to 14 | 13 |
| 98 | 3 | 6 | 84 to 86 | 15 to 17 | 14 |
| 97 | 4 | 7 | 81 to 83 | 18 to 20 | 15 |
| 96 | 5 | 8 | 78 to 80 | 21 to 23 | 16 |
| 95 | 6 | 9 | 73 to 77 | 24 to 28 | 17 |
| 93 to 94 | 7 to 8 | 10 | 67 to 72 | 29 to 34 | 18 |
| 91 to 92 | 9 to 10 | 11 | 56 to 66 | 35 to 45 | 19 |
| 89 to 90 | 11 to 12 | 12 | 51 to 55 | 46 to 50 | 20 |

This table indicates the maximum range that is the difference between the highest and lowest in germination percentages tolerable between replicates, allowing for random sampling variation only at 0.025 probabilities. To find the maximum tolerated range in any case, calculate the average percentage to the nearest whole number of the four replicates. If necessary,

From 100- seed replicates by combining the sub-replicates of 50 or 25 seeds which were closest together in the germinator. Locate the average in column 1 or 2 of the table and read off the maximum tolerated range opposite in column 3. This table indicates the tolerance to be used in deciding if two tests are compatible, allowing for random sampling variation only at 0.025 probability.

To determine if two tests are compatible, calculate the average percentage germination of the two tests to the nearest whole number and locate it in column 1 or 2 of

the Table. The tests are compatible if the difference between the germination percentages of the two tests does not exceed the tolerance in column 3 opposite.

**6.6.5 Compatibility of tests**

| Average percentage germination | | Tolerance |
|---|---|---|
| 1 | 2 | 3 |
| 98 to 99 | 2 to 3 | 2 |
| 95 to 97 | 4 to 6 | 3 |
| 91 to 94 | 7 to 10 | 4 |
| 85 to 90 | 11 to 16 | 5 |
| 77 to 84 | 17 to 24 | 6 |
| 60 to 76 | 25 to 41 | 7 |
| 51 to 59 | 42 to 50 | 8 |

**6.6.6 Reporting results**

The result of the germination test is calculated as the averages of 4 x 100 seed replicates. It is expressed as percentage by number of normal seedlings. The percentage is calculated to the nearest whole number. The percentage of abnormal seedlings, hard, fresh and dead seeds is calculated in the same way. These should be entered on the analysis certificate under appropriate space.

**6.7 Types of germination**

Two types of seed germination occur and neither appears to be related to seed structure. These two types can be illustrated by observing the germination of bean and pea seeds. Although these seeds are similar in structure, their germination patterns are quite different. Epigeal germination in beans and hypogeal in peas.

Epigeal germination

During germination, the cotyledons are raised above the ground. During root establishment the hypocotyl begins to elongate in an arch which breaks thro' the soil, pulling the cotyledon and enclosed plumule (epicotyl) thro' the ground and projecting them in the air. (eg.) bean, castor, cucurbits and other dicots and onion.

Hypogeal germination

During germination the cotyledons remain beneath the soil while the plumule pushes upward and emerges above the ground. Here the epicotyl (plumule) elongates (e.g.) Peas, grams, mango, grasses and many other species.

## 6.8 Quick viability test

This makes it possible to distinguish red coloured living parts of seeds from the colourless dead ones. Staining of seeds determines whether seeds are to be classified as viable. Completely stained seeds are viable, partially and comparatively unstained seeds are non-viable. This test is not valid for previously germinated seeds.

### 6.8.1 Method of Tetrazolium (TZ) testing

Testing sample

A representative sample of 50 (or) 100 seeds is usually sufficient. However 200 seeds, in replicates of 100 seeds are recommended.

Preparation of solutions

1% solution is used for seeds that are not bisected thro' the embryo, while 0.1% solution is used for seeds in which the embryo is bisected. The pH of the solution should be between 6 and 8 for best staining. If the pH of the water is not in the natural range, the TZ salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows:

Solution 1: Dissolve 9.078 g of $KH_2PO_4$ in 1000 ml of water.

Solution 2: Dissolve 11.876 g of $Na_2HPO_4 . 2H_2O$ in 1000 ml of water.

Take 400 ml of solution 1 and 600 ml of solution 2 and mix them together. In a litre of buffer solution prepared as above dissolve 10 gms of TZ salt. This gives 1 % TZ solution of pH 7.0. This may be further diluted to give lower concentrations. The solution should be stored in brown bottle to prevent deterioration from light.

Methods of preparation of TZ testing

The seeds are first prepared for staining, then stained and evaluated from light.

**Method 1**: Bisect longitudinally

Soak the seeds in water for 3 to 4 hours. Bisect the seeds by cutting longitudinally thus exposing the main structures of the embryo. Use1/2 of each of seed for testing. (e.g.) maize, sorghum, small grains, large seeded grasses.

**Method 2**: Bisect laterally

The seeds are cut laterally near the centre of the seed above the embryo. Place embryo end in TZ solution. (e. g) small seeded grasses.

**Method 3**: Pierce with needle

Puncture the seeds by piercing thro' the seed into the endosperm near the embryo but avoid injury to the embryo. (e.g) small seeded grass.

**Method 4**: Remove seed coat

Soak the seeds in water for 3-4 hours and then remove the seed coats and place the seeds in the TZ solution. In some crops like cotton a thin membrane adhering to the cotyledons is also removed in addition to the seed coat. (e.g) dicots with seed coats impermeable to tetrazolium

**Method 5**: Conditioning only

Seeds of soybeans and other large-seeded legumes may swell so rapidly and irregularly when placed directly in water or TZ solution that the seed coats burst. Hence, it is preferably to condition these seeds slowly in moist paper towels overnight before staining, so that they absorb moisture slowly without any damage to the seed. (e.g) Large seeded legumes

**Method 6**: No conditioning or preparation

Seed coats of these seeds are permeable to TZ and the embryos usually will stain without conditioning. (e.g.) Small seeded legumes

**Staining**

The prepared seeds should be placed in suitable container (small beakers, petridishes) and covered with TZ solution. Place the containers in an incubator at dark

warm conditions of 40°C. The staining time varies for different kinds of seeds, different methods of preparation, and different temperatures (>1 hr to 8 hrs). When sample has stained sufficiently the TZ solution should be discarded and the seed sample covered with water immediately. Seed samples can also be kept for 3 days at 10°C for interpretation.

Evaluation of samples

A normal stain appears cherry red.

**Monocots - Non viable**

1. All structures unstained
2. Shoot largely unstained.
3. Scutellar node unstained.
4. Major areas of coleoptile unstained
5. Central area of scutellum unstained
6. Insect, mechanical or other injuries causing essential structures non-functional.

**Dicot - Non viable**

1. Embryo completely unstained
2. More than extreme tip of radicle unstained
3. More than 1/2 of cotyledon tissue unstained
4. Deep seated necrosis at cotyledon and embryonic axis juncture or on radicle
5. Fractured radicle

## 6.9 DETERMINATION OF MOISTURE CONTENT

The moisture content of a seed sample is the loss in weight when it is dried. It is expressed as a percentage of the weight of the original sample. It is one of the most important factors in the maintenance of seed quality.

### 6.9.1 Methods of moisture determination

Air oven method

In this method, seed moisture is removed by drying at a specified temperature for a specified duration.

Moisture meters

Moisture meters estimate seed moisture quickly but the estimation is not as precise as by the air oven method.

Weight of the submitted sample

100 gm for species that have to be ground. 50 gm for all other species. The sample should be submitted in polythene bag of 700 guage.

**6.9.1.1 Air oven method for seed moisture estimation**

Materials required

Grinding mill

It should be constructed of non-absorbent material. It should grind evenly and should be operated at such a speed that during grinding, it should not cause heating of the ground material. Air currents that might cause loss of moisture must be reduced to a minimum. The finess of grinding should be adjustable.

Container

Containers of glass or non-corrosive metal (e.g. Stainless steel) should be used.

Oven

A good quality electric air oven with a thermostatic or electronic temperature control for maintaining temperature within = $1^0$C is required.

Desiccator

Analytical balance

Sieves : A set of wire mesh sieves with meshes of 0.5 mm, 1,0 mm and 4.0 mm.

Grinding

For some seeds (e.g. cereal and cotton), fine grinding is essential before the moisture content is determined. In such cases, at least 5% of the ground material should pass through a wire sieve with meshes of 0.5 mm and not more than 10 remain on a wire sieve with a mesh of 1.0 mm. For leguminous seeds, coarse grinding is recommended. At least 50% of the ground material shall pass through a wire sieve with meshes of 4.0 mm.

Pre drying

If the species is one for which grinding is necessary and the moisture content is more than 17 (or 10 in the case of soybean and 13% in rice) pre-drying before grinding is necessary. For this purpose, two 50 gm portions are weighed and placed on to open trays

at 130°C for 5-10 min. If seed moisture content is about 25.0% or more it should be pre-dried at 70°C for 2-5 hours, depending on the initial water content. The pre dried seeds should be kept in a closed desiccator for cooling. Then each of the duplicate quantities is weighed separately and about 20 g is ground. The ground material is then subjected to the moisture testing using an air oven as described below.

Moisture estimation

It should be carried out in duplicate on two independently drawn 5-10g working samples, weighed with an accuracy of 1 mg. Most species are dried for 1 hr at 130°C, cereals for 2 hrs (130°C) and maize for 4 hrs (130°C). Seeds containing high percentage of oil should be dried at 103°C for 17 hours.

**Steps:**

1. Empty container along with its cover should be weighed.
2. The submitted sample should be mixed thoroughly and two small portions of seed sample are weighed directly into the containers.
3. After weighing remove the cover or lid of the container and the open container should be kept in the oven which has already been heated to the prescribed drying temperature.
4. At the end of the drying period, container should be closed with its cover or lid.
5. The container should be transferred into a desiccator. The desiccator should be closed and the sample should be allowed to cool.
6. The sample should be weighed again and the moisture content may be calculated to one decimal place by the following formula.

$$m = \frac{m2 - m3}{m2 - m1} \times 100$$

Where m =seed moisture content.

m1 =weight of the empty container with its cover

m2= weight of the container with its cover and seeds before drying.

m3= weight of the container with its cover and seeds after drying.

The duplicate result of the determination may not differ by more than 0.2% otherwise the analysis should be repeated. If the material is pre-dried, the moisture content is calculated from the results obtained in the pre-dried and dried stages using the following formula.

$$M = S1 + S2 - \frac{S1 \times S2}{100}$$

M = moisture content
S1= moisture percentage lost in pre drying stage
S2= moisture percentage lost in drying stage.

### 6.9.2 moisture meters: universal Osaw digital moisture meters

The principle involved in these moisture meters is that wet grains are good conductors while dry grains are less conductors of electricity. So the moisture content is directly proportional to the electrical conductivity of the seed.

It consists of a compression unit to compress the sample to pre-determined thickness. The thickness setting is very easily read on a vertical and circular scale. The seed material on test is taken in a test cup and is compressed. Then press the push type switch till the reading comes in the display. Here no temperature reading and correlated dial are required. The computer versions of digital moisture meter automatically compensate for temperature corrections.

## 6.10 SEED HEALTH TESTING

It is done for determining the presence or absence of disease causing agents such as fungi, bacteria and viruses and insects in the seed samples. The pathogen may be carried with the seeds in the way.

**Admixture**

Pathogens are independent of seeds but accompany them. Ergot sclerotia are mixed with healthy seeds during threshing.

**External**

The pathogen may be present on seed surface as spores, oospores and chlamydospores as in case of karnal bunt of wheat, covered smut of barley, downy mildew of pearlmillet etc. By surface sterilization external seed borne diseases are killed.

Internal

Pathogens establish within the seed with definite relationship with seed parts.

**Procedure**

Working sample

The entire submitted sample, or a portion of it, depending on the test method may be used. Normally the working sample shall not be less than 400 pure seeds.

**Methods**

Examination without incubation

Such tests give no indication as to the viability of the pathogen.

Direct examination

The submitted sample, or a sub sample from it is examined, with or without a stereoscopic microscope and searched for ergots and other sclerotia, nematode galls, smut-balls, insects, mites and evidence of diseases and pests in seed or in inert matter.

**Examination of imbibed seeds**

The working sample is immersed in water or other liquid to make fruiting bodies, symptoms of pests etc., more easily visible, or to encourage the liberation of spores. After imbibition the seeds are examined either superficially or internally, preferably with the help of stereoscopic microscope.

Examination of organisms removed by washing

The working sample is immersed in water with a wetting agent or alcohol and shaken vigorously to remove fungal spores, hyphae, nematodes etc., intermingled with or adhering to the seeds. The excess liquid is then removed by filtration, centrifugation or evaporation and the extracted material examined with the help of a compound microscope.

Examination after incubation

After incubation for a specific period, the working sample is examined for the presence of symptoms of disease organisms, pests and evidence of physiological disturbances in the seeds and seedlings.

Blotters

These are used when pathogens are to be grown from the seeds or when seedlings are to be examined. The seeds with or without pretreatment are so spaced during incubation as to avoid secondary spread of organisms. Lighting is provided to stimulate sporulation of fungi when needed. Some pathogens can be identified without

magnification but a stereoscopic microscope or a compound is often helpful in identifying spores.

Sand, artificial composts and similar media can be used for certain pathogens. The seeds usually without pre-treatment, are sown suitably spaced in the medium so as to avoid secondary spread of organisms and then incubated in conditions favourable for symptom expression.

In seed spices, Altenaria blight caused by Alternaria burnsii in cumin and Alternaria blight as well as Ramularia blight in fennel are reported to be a partial seed borne diseases. For the pathogenic test of these diseases, the following methods are used.

1.Standard blotter paper method:

400 seeds of each sample will be taken at random. Used sterilized blotter paper at the bottom of each petri dish aseptically and moisturized by sterile water. Ten seeds are placed at equal distance in each petri dish and incubate at 20-25$^0$C under room temperature. The petri dishes will be examined after 4-7 days of incubation for the presence of seed borne mycoflora and count the percentage of infected seeds.

Agar plates are used to obtain identifiable growth of organisms from seeds. Precautions should be taken to ensure their sterilization. The seeds, normally after pre-treatment, are placed on the surface of sterilized agar and incubated. Characteristic colonies on the agar can be identified, either macroscopically or microscopically. Lighting is often useful and germination inhibitors may be used.

2. Agar plate Method

Four hundred seeds of each sample will be taken at random. Prepare PDA medium and ten seeds are placed at equal distance aseptically per petridishes containing 20 ml of PDA and incubate at 20-25$^0$c under room temperature.

The fungal growth on the seed will be examined regularlymand on seven days of incubation, count the percentage of infected seeds.Also transferred the individual fungal growth emerged from seeds on slant test tube by needle for pathogenecity. For sterilized condition, seeds will be treated with 0.1% $Hgcl_2$ solution for one minute followed by three washing of sterile distilled water, for unsterilized conditin, seed will be kept as such without any treatment. This method is more reliable than standard blotter paper method.

Examination of plants

Growing plants from seed and examining them for disease symptoms is sometimes the most practicable method for determining whether bacteria, fungi or viruses are present in the sample. Seeds from the sample under test may be sown or inoculum obtained from the sample may be used for infection tests with healthy seedlings or parts of plants. The plants must be protected from accidental infection from elsewhere and conditions may require careful control.

Other techniques

Specialized methods involving serological reactions, phage-plaque formation etc., have been developed for some disease organisms and may be used preferably in consultation with the seed pathologist.

Calculation and expression of results

Results are expressed as percentage by number of seeds affected or as number of organisms in the weight of sample examined. The result must be accompanied by statement of the test method used, including any pre-treatment applied, and of the amount of the sample or fraction examined. The absence of a statement concerning the health condition of the seed does not necessarily that the health condition is satisfactory.

## 6.11 seed storage

Seed storage is important to get adequate plant stands in addition to healthy and vigourous plants.

### Factors affecting seed longevity in storage

### Genetic factors

The storage is influenced by the kind/ variety of seeds. Some kinds are naturally short-lived (e.g.) onion soybeans, groundnut etc. Within a crop the storage period varies between varieties. Also the storage periods of hybrid and parent are differing.

Effects of weather

Fluctuating temperature during seed formation and maturity will affect seed storage pre harvest rain may also affect the viability.

**Pre harvest sanitation spray**

In pulses, insect infestation comes from field (e.g.) bruchids.

### Seed structures

The presence or absence of glumes (lemma and palea) in grasses influence the storage period. Husk, chaff or both have shown an inhibitory effect on the growth of mould and an increase in life span of cereals seeds. Generally small seeds escape injury; where as large seeds are more likely to be extensively damaged (e.g.) bean, lima bean and soybean.

### Initial quality of the seed

Seed lots having vigourous, undeteriorated seeds store longer than deteriorated lots.

### Environmental factors

### Moisture content

The amount of moisture in the seeds is the most important factor influencing seed viability during storage. Generally if the seed moisture content increases the storage life decreases. If seeds are kept at high moisture content the losses could be very rapid due to mould growth very low moisture content below 4% may also damage seeds due to extreme desiccation or cause hard-seediness in some crops.

Since the life of a seed largely revolves around its moisture content it is necessary to dry seeds to safe moisture contents. The safe moisture contents however depends upon storage length type of storage structure, kind / variety of seed, type of packing material used. For cereals in ordinary storage conditions for 12-18 months, seed drying upto 10% moisture content appears quite satisfactory. However, for storage in sealed containers, drying upto 5-8% moisture content depending upon particular kind may be necessary.

### Relative humidity and temperature during storage

Relative humidity is the amount of $H_2O$ present in the air at a given temperature in proportion to its maximum water holding capacity. Relative humidity and temperature are the most important factors determining the storage life of seeds. Seeds attain specific and characteristic moisture content when subjected to given levels of atmospheric humidity. This characteristic moisture content is called equilibrium moisture content. Equilibrium moisture content for a particular kind of seed at a given relative humidity tends to increase as temperature decreases.

Thus the maintenance of seed moisture content during storage is a function of relative humidity and to a lesser extent of temperature. At equilibrium moisture content there is no net gain or loss in seed moisture content.

**Temperature**

Temperature also plays an important role in life of seed. Insects and moulds increase as temperature increases. The higher moisture content of the seeds the more they are adversely affected by temperature. Decreasing temperature and seed moisture is an effective means of maintaining seed quality in storage. The following are thumb rules by Harrington are useful measures for assessing the effect of moisture and temperature on seed storage. These rules are as follows:

For every decrease of 1% seed moisture content, the life of the seed doubles. This rule is applicable between moisture content of 5-14%.

a. For every decrease of $5^oC$ in storage temperature the life of the seed doubles. This rules applies between $0^oC$ to $50^oC$.

b. Good storage is achieved when the percentage of relative humidity in storage environment and the storage temperature in degrees Fahrenheit add upto one hundred but the contribution from temperature should not exceed $50^oF$.

c. Nomograph

Roberts (172) developed formulae to describe the relationship between temperature, seed moisture content and period of viability. From these relationships it was possible to construct a seed viability nomograph. These nomographs are helpful in predicting the retention of seed viability in defined storage environment for a particular period or to determine combination of temperature and moisture content which will ensure the retention of a desired level of seed viability for a specific period.

d. Gas during storage

Increase in $O_2$ pressure decreases the period of viability.

$N_2$ and $CO_2$ atmosphere will increase the storage life of seeds.

**Microflora, insects and mites.**

The activity of all these organisms can lead to damage resulting in loss of viability.

The microflora activity is controlled by relative humidity, temperature and moisture content of seed.

## 6. 12 Seed treatment

Treated seeds with fungicides can be stored for longer periods. Fumigation to control insects will also help in longer period of storage.

**Fumigation**

Once the seed storage is free of completely free of insects, the most serious source of reinfestation is infested seed, which is brought in. Seed may be brought from the field already infested, or it maybe transferred from infested storage. Such infestation is controlled by fumigation. Fumigation is effective only in gas-tight storage. Numerous effective fumigants are available.

| *Fumigant* | *Dosage* | *Exposure period* |
|---|---|---|
| Methyl bromide | 16 to 32 mg / cubic meter | 24 hours |
| Hydrogen cyanide | 32 to 64 mg/cubic meter | 24 hours |
| Hydrogen phosphide | 5 to 10 tablets per tone of seed | 3 to 7 hours. |

It must be borne in mind that fumigation, particularly repeated fumigation, may seriously reduce the vigour and even the germination capacity of seeds. This is particularly true of seeds with high moisture content. Seeds with moisture content greater than 14 per cent should be dried to below this value before fumigation.

**Type of packing materials**

Moisture vapour proof containers can help in longer storage than the moisture pervious containers.

**Use of desiccants**

Desiccant like silica gel can maintain the moisture content in equilibrium with the relative humidity of 45%. It is kept @ 1 kg/10 kg of seeds. When the blue silica gel turns to pink colour it should be dried at 175$^{o}$C in oven and then again placed in the container.

## 6.13 SEED PACKING MATERIALS

Classification of packaging materials or containers as detailed below:

**Moisture and vapour pervious containers**

These containers allow entry of water in the form of vapour and liquid. These are suited for short-term storage. The seeds in these containers will attain seed equilibrium moisture with the surrounding atmosphere (e.g.) cloth bags, gunny bags, paper bags etc.

**Moisture impervious but vapour pervious containers**

These allow entry of water in the form of vapour and not in liquid. The seeds in these containers can't be carried over for long period in hot humid conditions. (e.g.) polythene bags of > 100 gauge thickness and urea bags.

**Moisture and vapour proof containers**

These containers will not allow entry of moisture in the form of liquid or vapour. These are used for long-term storage even in hot humid conditions if the seeds are sealed at optimum moisture content (e.g.) polyethylene bags of >700 gauge thickness, aluminum foil pouches, rigid plastics etc. Certified seeds of cereals, pulses and oil seeds are normally packed either in gunny bags or cloth bags. However, paper bag, aluminum foil pouches and polyethylene bags are used for packaging flower and vegetable seeds.

### 6.13.2 Types of storage requirements

The types of storages are based on the time of storage. It can be classified into 4 types as given below:

**Commercial seeds**: The largest storage need is for the storage of seed from harvest until planting. The storage period ranged from a few days to 8 or 9 months. Here seeds must be dried to a m.c of < 14% for starchy seeds and less than 11% for oil seeds.

**Carryover seeds**: About 20-25% of stored seeds may have to be carried over through one growing season to the second season. This storage period is usually between 1-year &11/2 years.

Seeds can be stored in steel bins with tight fitting lids or in moisture proof bags.

**Foundation seed stocks***:* This can be stored for several years, since reproducing foundation or stock seeds minimize genetic drift is. This seeds can be stored at expand about 25% and temperature at 30$^{o}$C or a relative humidity of 45% and temperature of 20$^{o}$C. This can be achieved by using a dehumidifier. Store the seeds with polythene bags of > 700 gauge thickness.

**Germplasm seeds**: These seeds are to be stored for many years. Basic requirements for such very long-term storage are coldest temperature and seed moisture content in

equilibrium with20-25% R.H. storage rooms can be, maintained at $5^oC$ and $10^oC$ and 30% R.H. Here the seeds should be dried to lower level.

**6.13.3 Agar plate method**

400 seeds of each sample will be taken at random. Prepare PDA medium and ten seeds are placed at equal distance aseptically per petridishes containing 20 ml of PDA and incubate at $20\text{-}25^0C$ under room temperature.

The fungal growth on the seed will be examined regularly and on seven days of incubation, count the percentage of infected seeds. Also transferred the individual fungal growth emerged from seeds on slant test tube by needle for pathogenicity. For sterilized condition, seeds will be treated with 0.1% $HgCl_2$ solution for one minute followed by three ashing of sterile distilled water, for un sterilized condition, seed will kept as such without any treatment. This method is more reliable than standard blotter paper method.

**Seed storage sanitation or godown sanitation**

1. Storage environment should be free from insects and rodents
2. Chemicals such as insecticides, fertilizers should not be stored along with seeds.
3. Storage room should be kept cool and dry.
4. Fumigation may be done whenever needed.
5. Use wooden pallets for arranging the bags in cris-cross manner for effective ventilation on all sides of the bags.
6. Seed bags should be stacked upto 6-8 tires depending upon density of seeds.
7. Restacking once in 3 months or less is important for prolonging seed viability.
8. Before storage disinfect the godowns by spraying malathion 50% E.C. @ lit /100 m area.
9. If old gunnies, cloth bags and containers are to be used these should be fumigated with aluminium Phosphide.
10. Size of the stack should be 30 x20 feet to facilitate fumigation under gas proof or polythene covers.

Periodical inspections should be carried out and control measures to be taken i.e. Malathion 50 E.C. @ lit /100 m should be applied in every 3 weeks. It must be borne in mind that fumigation; particularly repeated fumigation may seriously reduce the vigour

and even the germination capacity of seeds. Seeds with moisture content greater than 14% should be dried to below this value before fumigation.

### 6.13.4 Maintenance of viability in storage

1. Store well matures seeds.
2. Store normal coloured seeds.
3. Seeds should be free from mechanical injury.
4. Seeds should be free from storage fungi or microorganisms.
5. Seeds should not have met with adverse conditions during maturation.
6. Storage environment or godown should be dry and cool.
7. Seeds should be dried to optimum moisture content
8. Required relative humidity and temperature should be maintained during storage.
9. Seeds should be treated with fungicides before storage.
10. Storage godown should be fumigated to control storage insects, periodically.
11. Suitable packaging materials should be used for packing.

Format of Seed Analysis Report

**MINI SEED TESTING LAB ANALYTICAL REGISTER (To be maintained by ADSCN)**

| Sl.No. | Name and address of the applicant | Crop/Variety | Class of the seed | Details of the seed source | Date of expiry | Quantity of the lot | Date of receipt | Date of sampling |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

| Date of test | Normal seedlings | Analytical result abnormal seedlings | Hard seed | Dead seed | Date of Despatch or result | File No. | Seed Rate recommended | Initial of ADSCN |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

SEED SAMPLES AND SEED ANALYTICAL REGISTER (To be maintained by ADSCN)

| Sl.No. | Name and address of the Producer | Kind/Variety | Class | S.C.No. | Lot No. | Quantity of the lot | Date of test |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| Purity | OCS | Analytical test results | | | Hard seed | Moisture | Remarks |
| | | WS | ODV/GP | Germination | | | |

| Name of the SCO | Date of drawing sample by SCO | Date of receipt of Sample by ADSCN | ADSC Code No. | Date of receipt of analytical report | Kind of sample Fresh Resample Validation | STL Code No. | Date of communication of result to the producer | Initials of ADSCN |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

# SEED TESTING MANUAL
# FOR
# SEED SPICES CROPS

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# SEED TESTING MANUAL FOR SEED SPICES

## Introduction

Spices are fragrant products of plant origin used mostly for flavouring food and beverages. Among spices, seed spices comprise the single largest group with over 17 items coming under it. The important amongst this group are coriander, cumin, fennel, fenugreek, celery, ajowan seed, dill seed, aniseed etc. India is the largest producer of seed spices with a production of about 5.6 lakh tonnes of seed spices annually from an estimated area of about 8.5 lakh ha. This group has a prominent place in our national economy because of its large domestic consumption and growing demand for export. Being annual crops these are grown extensively in rotation with food crops and also as inter/mixed crops under rainfed/irrigated conditions. Seed spices are mainly cultivated in the states of Rajasthan and Gujarat with a sizeable area in the states of Madhya Pradesh, Chhatisgarh, Punjab, Uttar Pradesh, Tamil Nadu, Andhra Pradesh and Bihar.

The seed, a basic unit in agriculture plays a crucial role in boosting up the productivity and economy of the country. Without the use of good quality seed, the investments incurred on fertilizers, pesticides and water will not pay dividend which ought to be realized. There has been considerable coverage of area under seed spices but it's share towards production is less. It is due to low productivity levels obtained by the growers. One of the main reason contributing to low productivity is non-availability of quality seed of released varieties. So far 61 varieties of 9 different seed spices crops have been released but sufficient quantity of quality seed has not reached to farmers. Moreover, the seed spices crops have not received attention in national seed production policies. The information on seed certification of these crops has not been documented so far except fenugreek. The minimum seed certification standards and necessary guidelines on the quality seed production of seed spices are warranted by the seed producers and related agencies time and again. Hence in this compilation an attempt has been made to present the information for the benefit of seed spices producers and related agencies.

## 1. Seed quality parameters

There are four parameters considered important in quality seed production such as physical purity, germ inability, health and genetic purity. Physical Purity means that a seed sample should contain only seeds of a specific crop species and not any thing else(such as intert matter, weed seeds and other crop seeds). Germ inability indicates the capacity of seeds to germinate and emerge in the field as normal and vigorous seedlings. Seed health is related to seed borne diseases. The seed should be free from diseases. Genetic purity means that all plants in a population of a variety are genetically identical and population is homogeneous.

In fact the first and second parameter of quality seed determine the establishment of plant population per unit area. Poor physical purity and germ inability directly affect the population establishment and hence the poor yield. Similarly, the diseased seed may also directly affect the plant population by causing disease in the field condition. The fourth parameter, genetic purity is a necessary requirement to maximize the potential of improved varieties and prove the effectiveness of agriculture operation. In addition to above the quality seed should be uniform in size and should possess good lustre.

### 1.1 System of quality control

Quality control is an important component of the seed programme. In fact, the essence of any seed programme lies in the quality control. Seed quality control is a system which ensures to govern the quality to the seed through checks, certification and official regulations (legislation) for seed production. It is necessary that the quality of the seed should be of the highest possible standards. In other words, it may be inferred that the seed handled within the seed programme should be monitored to enuse that its quality is maintained during the production processes in such a way that the seed below certain acceptable standards in rejected. The seed quality control in itself ensures the interest of the farmers and to avoid hazards in the crop production. The seed quality control programme is based on following essential components.

1. Quality control of breeder seed

2. Seed certification
3. Seed legislation
4. Seed standards.
5. Seed testing.

It is the responsibility of government to enforce the measures for controlling the quality of the seed being marketed in the country. In India, seed quality control programme was initiated during 1963 with the establishment of the National Seed Corporation which was the single agency for production, certification and marketing of the seed throughout the country. However at a later stage, during, 1970's and 1980's, State Seed Certification Agencies were established in most of the states, which are responsible to carry forward the seed certification programme in the respective state.

Seed Act was passed in India during 1966 and amended in 1974. Indian Seeds Act is essentially a 'truthful' labelling act. The seed certification in India is voluntary. At present the Seed Act as well as Seed Certification in India is applicable to the, Notified kind/varieties. For the regulation of the Seed Act and the Seed Certification Programme in the country, two separate sets of the standards namely, Minimum labelling standards and Minimum Seed Certification Standards were prescribed during 1970 which have now been revised. Currently National Seed Policy is being revised in view of changing global scenario.

**1.2 Seed certification:**

The primary aim of seed certification is to check the crop from which the seed is produced and link this verification with agreed minimum standards of other important feature of seed lot. The object of the seed certification is to maintain and make available to the public through certification high quality propagating material of notified kind/varieties so grown and distributed as to ensure genetic identity and genetic purity. The certification standards in-force are Indian minimum seed certification standards and seed certification procedures together form the seed certification regulation. Seed of only

those varieties which are notified under section 5 of the seed Act shall be eligible for certification.

Out of different classes of seeds, the nucleus and breeder seeds are the highest pure seed, produced by the breeder under his direct supervision. Such class of seed does not require any certification. The other two classes' foundation seed and certified seed are the subsequent progenies of breeder seed, require certification by the authorised agencies as per the Minimum Seed Certification Standards.

**1.2.1 Phases of seed certification:**

There are six different phases of seed certification, where in the application for certification scrutinized and the basic seed source, it's class and other requirements of seed used for raising the seed crop are verified. Varieties evolved by the State Agricultural Universities and ICAR Institutes are notified under section 5 of Seed Act are only considered for seed certification process. Any person/company desire of producing certified seed shall register his name with concerned Seed Certification Agency. He has to under go following broad phases before the seed is produced and marketed.

1) Receipt and scrutiny of application.
2) Verification of seed sources, class and other requirement of the seed used for raising the seed crops.
3) Field inspection to verify conformity to the prescribed field standards.
4) Supervision at post harvest stages.
5) Seed sampling and analysis, including genetic purity test and seed health test.
6) Grant of certificate and certification tags, tagging and sealing.

**Seed certification standards:**

The seed quality control measures are stipulated to provide the standards for seed certification and seed labelling. The seed certification standards may refer to the field condition or may refer to the procedures in inspection of the harvested produces as well as manner of harvesting, transporting, processing and packing, unless a seed certification

agency keeps track of harvested produce until it is packed and sealed the identity of the lots can not be assured.

It is therefore necessary that seed certification agency should lay down standard for processing plant also. In addition, field and seed standards such as isolation distances, in separable other crops seeds, weeds, plants affected by seed borne diseases, genetic purity, percentage of pure seed, other crop and weed seeds, inert matter, moisture content, germination and insect damage should be prescribed for successful accomplishment of the certification. For the purpose of labelling the seed under the seed law enforcement programme, the standards for pure seed and germination is usually required. These standards will very according to the kind of seed. The standards should be reviewed at timely intervals in order to make term realistic.

The general seed certification standards are applicable to all crops, which are eligible for certification and with field and standards for the individual crops shall constitute the minimum seed certification standard. The seed spices crops so far considered as under exploited crops and thus have not received place in the compilation "Minimum Seed Certification Standards". In the changing paradigm of agricultural production, the seed spices crops have emerged as important crops. The quality seed of seed spices have become the pre-requisite in successful raising of these crops require information on seed certification standard particularly the field standards and seed standards.

**a) Field standards on seed spices crops :**

The necessary field standards consists of isolation distance, field inspection stages and specific requirements showing maximum permitted limits of off types, other crops and diseased plants are mentioned here.

i) Land requirement: The land intended for seed production must be free from volunteer plants. The field must have not been under same crop in pervious season.
ii) Isolation distance: The seed spices crops coriander, cumin, fennel and ajowain belongs to family Apiaceae and are mostly cross pollinated in nature. The cross pollination occurs mostly through insects (entomophilous). The coriander and cumin are considered as often cross pollinated whereas fennel, ajowain and dill are

cross pollinated crops. The fenugreek is a self pollinated in nature. The isolation distance to be kept between the fields of two varieties to produce foundation and certified seed, respectively are given in Table 1.

Table:1 Isolation distance for seed spices crops

| S.No. | Name of crops | Distance in meters | |
|---|---|---|---|
| | | Foundation seed | Certified seed |
| 1 | Fenugreek (highly self pollinated) | 50 | 25 |
| 2 | Coriander (Cross-pollinated) | 800 | 400 |
| 3 | Cumin (Cross pollinated) | 800 | 400 |
| 4 | Fennel (Cross pollinated) | 1000 | 800 |
| 5 | Ajowain (Cross pollinated) | 1000 | 800 |
| 6 | Dill (Cross pollinated) | 1000 | 800 |

**iii) Field Inspections:**

The field inspection means to verify those factors which can cause irreversible damage to the genetic purity or seed health. Field inspections are to be conducted withour prior notice to the seed producer by the persons who have been so authorised by the seed certification Agency. Normally the inspection team should comprise the seed productions officers of national seed corporation, state seed certification Agency and Seed Spices breeder. The crop stages at which inspection is to be done very with the nature of crop. A minimum three field inspection is made at following stages:

1. Before flowering: It is the first inspection stage done before flowering to determine isolation, planting ratio, volunteer plants, designated diseases and relevant factor.

2. At flowering and seed setting: This second inspection is done during flowering to rouge early flower type, plants showing different flowering behaviour, off types, designated diseases and other relevant factors.
3. Before harvesting: The fourth inspection is done at maturity to verify designated disease, true nature of plant, umbels, characteristic of seeds and confirmation of removal off types.

During field inspections, the rouging should be done at different stages by critically monitoring the seed crop at different stages. The undesirable plant from seed crop are removed on the basis of visual inspection in the field. The plant deviating from crop variety in the expression of morphological characters may be identified in the field, classified as off types also included under rogue. The process of roguing to typical plants should be completed before initiation of flowering so that honeybees could not visit done.

The specific requirement for seed crop of seed spices showing maximum permitted limits for off types, objectionable weeds, and designated diseases are given in Table 2. The objectionable weeds and designated diseases as reported for various seed spices crops are given here:

**Objectionable weeds in seed spices:**

Fenugreek: *Melilotus* (senji)

Cumin: *Plantago pumila* (Jiri)
Ajowain : *Ammi majus*
*Nigella : Nigella damescena*

*Designated seed borne and other diseases in seed spices:*

Fenugreek: Root rot (*Rhizoctonia solani*), downy mildew (*Peronospora trigonella*), leaf spot (*Cercospora traversions*), powdery mildew (*Erisiphae* spp.)
Coriander: Fusarium wilt (*Fusarium oxysporum* f.sp. *coriandrii*), stem gall (*Protomyces macrosporus*), downy mildew, powdery mildew (*Erisiphae* spp.)
Cumin: Fusarium wilt (*Fusarium oxysporum* f.sp. *cuminaii*), Cumin blight (*Alternaria cuminaii*), powdery mildew (*Erisiphae* spp.)
Fennel: Alternaria blight (*Alternaria tenius*) and Ramularia blight (*Ramularia foeniculaii*)
Ajowain : Collar and root rot (*Scleroticum rolfsii*), Blight (*Alternaria* spp.)
Dill: Root rot (*Fusarium oxysporum)*
Nigella: Root rot (*Fusarium oxysporum)*
Anise: Alternaria blight, powdery mildew
Celery: Yellow virus
Caraway: Alternaria blight, powdery mildew

Table 2: Specific seed requirement for seed spices crops

| S.No. | Name of Crops | Maximum permitted limits (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Foundation seed | | | Certified seed | | |
| | | Off type | Objection able weed | Diseased Plants | Off Type | Objection able weed | Diseased Plants |
| 1 | Fenugreek | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| 2 | Coriander | 0.10 | - | 0.10 | 0.50 | - | 0.50 |
| 3 | Cumin | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| 4 | Fennel | 0.10 | - | 0.10 | 0.20 | - | 0.50 |
| 5 | Ajowain | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| 6 | Dill | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| 7 | Anise | 0.10 | 0.01 | 0.10 | 0.20 | - | 0.50 |
| 8 | Celery | 0.10 | 0.01 | 0.10 | 0.20 | - | 0.50 |
| 9 | Caraway | 0.10 | 0.01 | 0.10 | 0.20 | 0.02 | 0.50 |
| 10 | Nigella | 0.10 | 0.01 | 0.10 | 0.50 | 0.02 | 0.50 |

**1.3 Seed Standards in seed spices crops**:

The seed spices crop meeting field standards for certification should be harvested,threshed and processed as per guidelines issued by the certification agency. Soon after completion of seed processing the composite sample is taken for analysis of the seed standards such as percentage of pure seed, inert matter, other crop seed, total weed seed, germination and moisture. In order to ensure quality seed production, it is necessary to strictly follow the seed standards. The prescribed permittable seed standards limits of seed spices crops is given in Table 3. On receipt of Seed Analysis Report and the results of grow-out-test, the packing, tagging, sealing follows the issuance of certificate.

Table 3: Seed Standards for Seed Spices Crops

| S. No. | Name of Crop | Seed Standard(Percent) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pure seed (minimum) | | Inert matter (Maximum) | | Other crop seed (Maximum) | | Total weed seed (Maximum) | | Germnation (%) | | Moisture % | |
| | | F | C | F | C | F | C | F | C | F | C | F | C |
| 1 | Fenugreek | 98 | 98 | 2 | 2 | 0.10 | 0.20 | 0.10 | 0.20 | 70 | 70 | 8 | 8 |
| 2 | Coriander | 98 | 97 | 2 | 3 | 0.10 | 0.20 | 0.10 | 0.20 | 65 | 65 | 10 | 10 |
| 3 | Cumin | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 65 | 65 | 8. | 8 |
| 4 | Fennel | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 70 | 70 | 8 | 8 |
| 5 | Ajowain | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 65 | 65 | 8 | 8 |
| 6 | Dill | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 70 | 70 | 8 | 8 |
| 7 | Anise | 95 | 95 | 2 | 3 | 0.10 | 0.20 | 0.10 | 0.20 | 65 | 65 | 10 | 10 |
| 8 | Celery | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 65 | 65 | 8. | 8 |
| 9 | Caraway | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 70 | 70 | 8 | 8 |
| 10 | Nigella | 95 | 95 | 5 | 5 | 0.05 | 0.10 | 0.10 | 0.20 | 65 | 65 | 8 | 8 |

Note : 1. Insect damage seeds should not be more than 0.5% in each sample.
2. The moisture % for vapour proof container should be 2% less than the prescribed limit.

## 1.4 General Principles of seed testing

Seed testing procedures are developed to assess the quality of seed before it is sown. The quality of seed ultimately determines it's planting value and is important to the farmers and seed producers as well as seed organizations for labeling and marketing as well.

Seed testing includes procedures for evaluating the planting value of seeds and has been developed to achieve the following objectives:

- To determine the quality i.e. the suitability for sowing
- To identify seed quality problems and their probable cause
- To determine the need for drying and processing and the procedures to be followed
- To determine the quality standards for labeling specifications
- To establish the quality and provide basis for pricing and marketability

The seed testing rules focus mainly on following parameters.

- Seed sampling
- Purity analysis
- Germination test
- Biochemical test for viability
- Seed health testing
- Determination of moisture content
- Seed vigour test
- Testing of genuineness of cultivars

## 1.4 SEED SAMPLING

**Taking representative sample:**

The object of sampling is to obtain a sample of a suitable size for tests. A grain spear can be used to collect a representative sample of the grain. These samples can give a biased indication of seed viability. The objective is to obtain sample from a seed lot by taking a small portion at random from different places and combining them. Contact a seed-testing laboratory that can recommend a sampling procedure for your particular situation.

The first step in seed testing is sampling.

***Seed lot***: It is a uniformly blended specified quantity of seed physically identifiable in respect of which seed lot certificate may be issued either in bag or in bulk. The maximum quantity based on seed size of seed spices is 1,0000 kg per lot.

**Each sample should carry following informations:**

- Name and address
- Kind of seed and variety
- Year of harvest
- Type of test required (e.g. germination, purity or tetrazolium), and then forward the sample to be tested to the seed laboratory of your choice.

**Sampling intensity**

For seed lots in bags (or container of similar capacity that are uniform in size)

| | |
|---|---|
| Up to 5 containers | Sample each container but never < 5 primary samples |
| 6-30 containers | Sample atleast one in every 3 containers but never < than 5 primary samples |
| 31-400 containers | Sample atleast one in every 5 containers but never < than 10 primary samples |

When the seed is in small containers such as tins, cartons or packets a 100 kg weight is taken as the basic unit and small containers are combined to form sampling

units not exceeding these weight e.g. 20 containers of 5 kg each. For sampling purpose each unit is regarded as one container.

**For seeds in bulk**

| | |
|---|---|
| Up to 500 kg | At least 5 primary samples |
| 501 to 3000 kg | One primary sample for each 300 kg, but not less than 5 primary samples |
| 3001-20,000 kg | One primary sample for each 500 kg, but not less than 10 primary samples |

### 1.4.3 METHODS OF SAMPLING

**Hand sampling**

In this method it is very difficult to take samples from the deeper layers of bag. To over come this, bags are emptied completely or partly and then seed samples are taken. While removing the samples from the containers, care should be taken to close the fingers tightly so that no seeds escape. Thus using triers for sampling is best method of sampling from bulks.

**Sampling with triers**

Bin samplers or nobbe trier or stick/sleeve triers can be used for taking samples from bags or from bulk.

Bin samplers: Used for drawing samples from the lots stored in the bins.

Nobbe trier: This is suitable for sampling seeds in bag, not in bulk.

Sleeve type triers or stick triers: It is the most commonly used trier for sampling. There are two types *viz., w*ith compartments and without compartments. This trier is used for drawing seed samples from the seed lots packed in bags or in containers.

**Weight of submitted sample**

The minimum weight for submitted samples for various tests is as follows:

**Moisture test**

For moisture determination submitted sample of 100 grams for those species that have to be ground and 50 grams for all other species should be taken.

**For verification of species and cultivar**

| Crop | Lab only (g) | Field plot & lab (g) |
|---|---|---|
| Coriander, fennel, Fenugreek | 500 | 1000 |
| cumin, , dill, anise, nigella | 200 | 400 |
| Celery, ajowan | 100 | 200 |

**For other tests like purity and count of other species**

| Crop | Size of seed lot (kg) | Size of submitted sample (g) | Size of working sample for purity (g) | Size of working sample for count of other species (g) |
|---|---|---|---|---|
| Coriander, Fennel, Fenugreek | 20,000 | 400 | 50 | 500 |
| cumin, dill, anise, nigella, celery, ajowan | 10,000 | 180 | 18 | 180 |

The samples taken may be packed in bags, sealed and marked for identification. For moisture testing the samples should be packed separately in moisture proof polythene bag and kept in the container along with submitted samples.

### 1.4.5 Information to accompany the sample

Date, Kind, Variety, Class of seed, Lot No., and Quantity of seed in lot (kg), Sender's name and address. Test(s) required: (1) Purity (2) Germination and (3) Moisture.

### 1.4.6 Types of sample used in seed testing lab

Service sample : Sample received from the farmers.
Certified sample : Sample received from certification agencies or officers.
Official sample : Sample received from the seed inspectors.

## 1.5 MIXING AND DIVIDING OF SEEDS

**Mechanical dividing**

The reduction of sample size is carried out by the mechanical dividers suitable for all seeds except for chaffy and fuzzy seeds.

**Soil divider:** It is suitable for large seeds and chaffy seeds eg. Fennel, fenugreek, dill, cumin etc.

**Random cups method**

This method is suitable for seeds like celey and ajowan. Six to eight small cups are placed at random on a tray. After a preliminary mixing, the seed is poured uniformly over the tray. The seeds that fall into the cup are taken as the working sample.

**Spoon method**

This is suitable for samples of single small seeded species. Sufficient portions of seed are taken to estimate a working sample of approximately but not less than the required size.

## 1.5 PHYSICAL PURITY ANALYSIS

Purity test

A purity test is the analysis of a representative sample of the seed sample for pure seed, weed seed, other crop seed and inert matter. Any prohibited matter (Seeds and live insects) found during the purity analysis are identified and entered on the report. Seed must not be sold until prohibited material is removed from the seed lot. The purity test does not cover the genetic purity of the sample.

**Pure seed*:*** The seeds of kind / species stated by the sender. It includes all botanical varieties of that kind / species. Immature, undersized, shrivelled, diseased or germinated seeds are also pure seeds. It also includes broken seeds, if the size is > 1/2 of the original size.

***Other crop seed*:** It refers to the seeds of crops other than the kind being examined.

***Weed seed* :** It includes seeds of those species normally recognized as weeds or specified under seed act as a noxious weed.

***Inert matter*:** It includes seed like structures, stem pieces, leaves, sand particles, stone particles, pedicles and chaffs.

**Method:**

The Working sample:

The purity analysis is done on the working sample of prescribed weight drawn from submitted samples. The analysis may be made on one working sample of the prescribed weight, or on two sub-samples of at least half this weight, each independently drawn.

Weighing of the working sample

The number of decimal places to which the working sample and the components of the working sample should be weighed as below:

| Weight of the working sample in gram | Number of decimal places required | Example |
|---|---|---|
| <1 | 4 | 0.7534 |
| 1-9.999 | 3 | 7.534 |
| 10-99.99 | 2 | 75.34 |
| 100-999.9 | 1 | 753.4 |
| 1000 or more | 0 | 753 |

**Physical separation:**

The working sample after weighing is separated into its components *viz.*, pure seed, other crop seed, weed seed and inert matter.

**Method of purity separation:**

Place the sample on the purity work board after sieving / blowing operations and separate into pure seed, other crop seeds, weed seeds and inert matter. After separation identify each kind of weed seeds, other crop seeds as to genus and species. The names and number of each are recorded. The type of inert matter present should also be noted.

All the four components must be weighed to the required number of decimal places. The percentage of the components is determined as follows:

$$\text{\% of component} = \frac{\text{Weight of individual component}}{\text{Total weight of all components}} \times 100$$

If there is a gain or loss between the weight of the original samples and the sum of all the four components is in excess of one percent, another analysis should be made.

Duplicate tests

Analysis result near the borderline in relation to the seed standards, one more test is done and the average is reported. However, if a duplicate analysis is made of two half

samples, or whole samples, the difference between the two must not exceed the permissible tolerance.

Determination of inseparable other crop seeds and objectionable weed seeds (by Number /kg)

Whole submitted sample is used and the number per kg may be calculated and reported even if the working sample is less than a Kg.

Determination of Other Distinguishable Varieties **(ODV)**

Ten times, the size of working sample is used. It is determined based on the morphological characters of the seeds. The authentic samples should be available for comparison. The number of ODV should be calculated and reported as No./kg of seeds.

Calculation of results:

The % by weight of each of the component should be calculated to one decimal place and the total of all components must be 100. Components of < 0.05% shall be reported as Trace. If the result for a components is nil, this must be shown as '-0.0-' in the appropriate space.

**Equipment used for purity analysis**

Seed Blower

There are 2 plastic columns one for larger seeds and the other for smaller seeds. The plastic column is provided with a semi-circular outlet where the terminal velocity of wind can be adjusted. A time clock is also provided for the automatic running of the bowler. The inert matter is separated by stratification using the terminal velocity of air.

Purity work board

This is used for effective separation of different components. At the centre of the board, there is an illumination by which the emptiness of the seed is easily identified.

**1.6 GERMINATION TEST**

Germination tests shall be conducted with the pure seed fraction. A minimum of 400 seeds are required in four replicates of 100 seeds each or 8 replicates of 50 seeds each depending on the size of the seeds and size of containers of substrate. The test is conducted under favourable conditions of moisture, temperature, suitable substratum and

light if necessary. No pretreatment to the seed is given except for those recommended by ISTA.

Requirement for laboratory germination test

Medium

The medium serves as moisture reservoir and provides a surface or medium for which the seeds can germinate and the seedlings grow. The commonly used substrata are paper, sand and soil.

Most widely used paper substrates are filter paper, blotter or towel (kraft paper). It should have capillary movement of water, at vertical direction (30 mm rise / min.) .It should be free from toxic substances and free from fungi or bacteria. It should hold sufficient moisture during the period of test. The texture should be such that the roots of germinating seedlings will grow on and not into the paper.

Methods

Top of paper (TP): Seeds are placed on one or more layers of moist filter paper or blotter paper in petridishes. The petridishes are covered with lid and placed inside the germination cabinet. This is suitable for those seeds, which require light.

Between paper (BP): The seeds are germinated between two layers of paper.

Roll towel method: The seeds are placed between two layers of paper and rolled in towels. The rolled towels are placed in the germinator in an upright position.

Inclined plate method: Germination on glass plate with germination paper and kept at an angle of $45^{o}$.

**Sand**

Size of sand particle: Sand particles should not be too large or too small. The sand particles should pass through 0.80 mm sieve and retained by 0.05 mm sieve.

Toxicity: Sand should not have any toxic material of any pathogen. If any pathogen is found, then the sand should be sterilized in an autoclave.

Germination tray: When we use the sand, germination trays are used to carry out the test. The normal size of the tray is 22.5 x 22.5 x 4 cm. The tray may be either zinc or stainless steel.

**Method of seed treatment**

Seeds in sand (s): Seeds are planted in a uniform layer of moist sand and then covered to a depth of 1 cm to 2 cm with sand.

Top of sand (TS): Seeds are pressed into the surface of the sand.

Spacing: We must give equal spacing on all sides to facilitate normal growth of the seedling and to avoid entangling of seed and spread of disease.Spacing should be 1-5 times the width or diameter of the seed.

Water: The amount of water to be added to the sand will depend on size of the seed. For cereals, except maize, the sand can be moistened to 50% of its water holding capacity. For large seeded legumes & maize sand is moistened to 60% WHC.

**Soil**

Should be non-caking, free from any large particles. It must be free from weed seeds, bacteria, fungi, nematode or toxic substances. Soil is not recommended for reuse.

Temperature: Required temperature is maintained (most seeds germinate between 20-30$^{o}$C)

Light: Light should be provided for seeds requiring light for germination (e.g.) celery.

### 1.6.1 Germination apparatus

Germination cabinet / Germinator: This is closed chamber where in temperature and relative humidity is controlled. We can maintain the required temperature.

Walk in room germinator: It works with same principles as that of germinator. This is modified chamber of larger one and the worker can enter into it and evaluate the seedlings. Provisions are made to maintain the temperature and relative humidity. This is used widely in practice.

Counting board: This is used for accurate counting and spacing of seeds. This consists of 2 plates. The basal one is stationary and top one is movable. Both top and basal plates are having uniform number of holes *viz*., 50/100, when the plates are in different position. After taking the sample, the top plate is pulled in such a way that the holes are in one line so that the fixed numbers of seeds fall on the substratum.

Vacuum counter: Consists of head, pipe and wall. There are plates of 50 or 100 holes which can be fitted to the head. When vacuum is created the plate absorbs seeds and once the vacuum is released the seeds fall on the substrate.

Impression board: Made of plastic / wood with 50 or 100 holes per pins. Here the knobs are arranged in equal length and space. By giving impression on the sand it makes uniform depth and spacing for seed.

**GERMINATION REQUIREMENTS FOR DIFFERENT CROPS**

| Crop | Substratum | Temp. ($^0$ C) | Light | First count days | Final Count days | Recommendation for breaking dormancy |
|---|---|---|---|---|---|---|
| Coriander (*Corian*dum *sativum* I) | TP,BP, S | 20-30, 20 | - | 5 | 10 | - |
| Fennel (*Foeniculam vulgar*e Mill) | TP,BP,S | 20-30 | - | 5 | 14 | - |
| Fenugreek (*Trigonella foenumgraecum* L) | TP,BP,S | 20-30, 20 | - | 4 | 14 | - |
| Cumin (*Cuminum cyminum*) | TP,BP,S | 20-30, 20 | - | 5 | 7 | - |
| Ajowan (*Trachyspermum ammi*) | TP, | 20-30, 20 | - | 5 | 14 | - |
| Dill (*Anethum graveolens, A. sowa*) | TP,BP,S | 20-30, 20 | - | 5 | 7 | - |
| Anise (*Pimpenella anisum*) | TP,BP,S | 20-30, 20 | - | 5 | 7 | - |
| Celery (*Apium graveolens)* | TP | 20-30 | L | 9 | 24 | Pre chill, $KNO_3$ , Light |

TP- TOP of paper
BP- Between paper
S- Sand

**Seedling evaluation**

ISTA classified the seedlings into different categories based on the development of essential structures.

**Categories of seedlings**

**Normal seedlings**

Seedlings which show the capacity for continued development into normal plant when grown in favourable conditions of soil, water, temperature and light.

**Characters of normal seedlings**

j. Seedlings with intact root and shoot system showing no visible deformities.
k. A well-developed root system with primary root.
l. A well-developed shoot axis consists of elongated hypocotyl in seedlings of epigeal germination.
m. A, well-developed epicotyl in seedlings of hypogeal germination.
n. One cotyledon in monocotyledons and two in dicotyledons
o. A, well-developed plumule in dicotyledons.
p. Seedlings with following slight defects are also taken as normal seedlings. Primary root with limited damage but well developed secondary roots.
q. Seedlings with limited damage or decay to essential structures but no damage to conducting tissue.
r. Seedlings, which are decayed by pathogen but it, is clearly evident that the parent seed is not the source of infection.

**Abnormal seedlings**

Seedlings which do not show the capacity for continued development into normal plant when grown in favourable conditions of soil, water, temperature and light.

**Type of abnormal seedlings:**

Damaged seedlings: Seedlings with any one of the essential structures are missing or badly damaged so that the balanced growth is not expected. Seedlings with no cotyledons, with splits, cracks and lesions on essential structures and without primary root.

Deformed seedlings: Weak or unbalanced development of essential structures such as spirally twisted or stunted plumule or hypocotyl or epicotyl, swollen shoot, stunted roots etc.,

Decayed seedlings: Seedlings with any one of the essential structures showing diseases or decayed symptoms as a result of primary infection from the seed which prevents the development of the seedlings.

**Hard seeds**

Seeds which do not absorb moisture till the end of the test period and remain hard (e.g.) seeds of leguminosae.

**Fresh ungerminated seeds**

Seeds which are neither hard nor have germinated but remain firm and apparently viable at the end of the test period.

**Dead seeds.**

Seeds at the end of the test period are neither hard nor fresh or have produced any part of the seedlings. Often dead seeds collapse and a milky paste come out, when pressed at the end of the test.

**Retesting**

If the results of a test are considered unsatisfactory it shall not be reported and a second test shall be made by the same method or by alternative method under the following circumstances.

d. Replicates performance is out of tolerance
e. Results being inaccurate due to wrong evaluating of seedlings or counting or errors in test conditions.
f. Dormancy persistence or phytotoxicity or spread of fungi or bacteria. The average of the two tests shall be reported.

**Use of tolerances**

The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances. To decide if two test results of the same sample are compatible again the tolerance table is used.

**Maximum tolerated ranges between replicates**

| Average percentage germination | | Maximum range | Average percentage germination | | Maximum range |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 1 | 2 | 3 |
| 99 | 2 | 5 | 87 to 88 | 13 to 14 | 13 |
| 98 | 3 | 6 | 84 to 86 | 15 to 17 | 14 |
| 97 | 4 | 7 | 81 to 83 | 18 to 20 | 15 |
| 96 | 5 | 8 | 78 to 80 | 21 to 23 | 16 |
| 95 | 6 | 9 | 73 to 77 | 24 to 28 | 17 |
| 93 to 94 | 7 to 8 | 10 | 67 to 72 | 29 to 34 | 18 |
| 91 to 92 | 9 to 10 | 11 | 56 to 66 | 35 to 45 | 19 |
| 89 to 90 | 11 to 12 | 12 | 51 to 55 | 46 to 50 | 20 |

This table indicates the maximum range that is the difference between the highest and lowest in germination percentages tolerable between replicates, allowing for random sampling variation only at 0.025 probabilities. To find the maximum tolerated range in any case, calculate the average percentage to the nearest whole number of the four replicates. If necessary,

From 100- seed replicates by combining the sub-replicates of 50 or 25 seeds which were closest together in the germinator. Locate the average in column 1 or 2 of the table and read off the maximum tolerated range opposite in column 3. This table indicates the tolerance to be used in deciding if two tests are compatible, allowing for random sampling variation only at 0.025 probability.

To determine if two tests are compatible, calculate the average percentage germination of the two tests to the nearest whole number and locate it in column 1 or 2 of the Table. The tests are compatible if the difference between the germination percentages of the two tests does not exceed the tolerance in column 3 opposite.

**Compatibility of tests**

| Average percentage germination | | Tolerance |
|---|---|---|
| 1 | 2 | 3 |
| 98 to 99 | 2 to 3 | 2 |
| 95 to 97 | 4 to 6 | 3 |
| 91 to 94 | 7 to 10 | 4 |
| 85 to 90 | 11 to 16 | 5 |
| 77 to 84 | 17 to 24 | 6 |
| 60 to 76 | 25 to 41 | 7 |
| 51 to 59 | 42 to 50 | 8 |

**Reporting results**

The result of the germination test is calculated as the averages of 4 x 100 seed replicates. It is expressed as percentage by number of normal seedlings. The percentage is calculated to the nearest whole number. The percentage of abnormal seedlings, hard, fresh and dead seeds is calculated in the same way. These should be entered on the analysis certificate under appropriate space.

**1.7 Quick viability test**

This makes it possible to distinguish red coloured living parts of seeds from the colourless dead ones. Staining of seeds determines whether seeds are to be classified as viable. Completely stained seeds are viable, partially and comparatively unstained seeds are non-viable. This test is not valid for previously germinated seeds.

**Method of Tetrazolium (TZ) testing**

Testing sample

A representative sample of 50 (or) 100 seeds is usually sufficient. However 200 seeds, in replicates of 100 seeds are recommended.

Preparation of solutions

1% solution is used for seeds that are not bisected thro' the embryo, while 0.1% solution is used for seeds in which the embryo is bisected. The pH of the solution should be between 6 and 8 for best staining. If the pH of the water is not in the natural range, the TZ salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows:

Solution 1: Dissolve 9.078 g of $KH_2PO_4$ in 1000 ml of water.

Solution 2: Dissolve 11.876 g of $Na_2HPO_4 . 2H_2O$ in 1000 ml of water.

Take 400 ml of solution 1 and 600 ml of solution 2 and mix them together. In a litre of buffer solution prepared as above dissolve 10 gms of TZ salt. This gives 1 % TZ solution of pH 7.0. This may be further diluted to give lower concentrations. The solution should be stored in brown bottle to prevent deterioration from light.

Methods of preparation of TZ testing

The seeds are first prepared for staining, then stained and evaluated from light.

**Method 1**: Bisect longitudinally

Soak the seeds in water for 3 to 4 hours. Bisect the seeds by cutting longitudinally thus exposing the main structures of the embryo. Use1/2 of each of seed for testing.

**Method 2**: Bisect laterally

The seeds are cut laterally near the centre of the seed above the embryo. Place embryo end in TZ solution.

**Method 3**: Pierce with needle

Puncture the seeds by piercing thro' the seed into the endosperm near the embryo but avoid injury to the embryo.

**Method 4**: Remove seed coat

Soak the seeds in water for 3-4 hours and then remove the seed coats and place the seeds in the TZ solution. In some crops like cotton a thin membrane adhering to the cotyledons is also removed in addition to the seed coat. (e.g) dicots with seed coats impermeable to tetrazolium

**Method 6**: No conditioning or preparation

Seed coats of these seeds are permeable to TZ and the embryos usually will stain without conditioning. (e.g.) Small seeded legumes

**Staining**

The prepared seeds should be placed in suitable container (small beakers, petridishes) and covered with TZ solution. Place the containers in an incubator at dark warm conditions of 40$^o$C. The staining time varies for different kinds of seeds, different methods of preparation, and different temperatures (>1 hr to 8 hrs). When sample has

stained sufficiently the TZ solution should be discarded and the seed sample covered with water immediately. Seed samples can also be kept for 3 days at 10$^{o}$C for interpretation.

Evaluation of samples

A normal stain appears cherry red.

**Non viable**

6. Embryo completely unstained
7. More than extreme tip of radicle unstained
8. More than 1/2 of cotyledon tissue unstained
9. Deep seated necrosis at cotyledon and embryonic axis juncture or on radicle
10. Fractured radicle

## 1.8 DETERMINATION OF MOISTURE CONTENT

The moisture content of a seed sample is the loss in weight when it is dried. It is expressed as a percentage of the weight of the original sample. It is one of the most important factors in the maintenance of seed quality.

### 6.9.1 Methods of moisture determination

Air oven method

In this method, seed moisture is removed by drying at a specified temperature for a specified duration.

Moisture meters

Moisture meters estimate seed moisture quickly but the estimation is not as precise as by the air oven method.

Weight of the submitted sample

100 gm for species that have to be ground. 50 gm for all other species. The sample should be submitted in polythene bag of 700 guage.

**Air oven method for seed moisture estimation**

Materials required

Grinding mill

It should be constructed of non-absorbent material. It should grind evenly and should be operated at such a speed that during grinding, it should not cause heating of the ground material. Air currents that might cause loss of moisture must be reduced to a minimum. The finess of grinding should be adjustable.

Container

Containers of glass or non-corrosive metal (e.g. Stainless steel) should be used.

Oven

A good quality electric air oven with a thermostatic or electronic temperature control for maintaining temperature within = $1^0$C is required.

Desiccator

Analytical balance

Sieves : A set of wire mesh sieves with meshes of 0.5 mm, 1,0 mm and 4.0 mm.

Grinding

For some seeds (e.g. cereal and cotton), fine grinding is essential before the moisture content is determined. In such cases, at least 5% of the ground material should pass through a wire sieve with meshes of 0.5 mm and not more than 10 remain on a wire sieve with a mesh of 1.0 mm. For leguminous seeds, coarse grinding is recommended. At least 50% of the ground material shall pass through a wire sieve with meshes of 4.0 mm.

Pre drying

If the species is one for which grinding is necessary and the moisture content is more than 17 (or 10 in the case of soybean and 13% in rice) pre-drying before grinding is necessary. For this purpose, two 50 gm portions are weighed and placed on to open trays at 130°C for 5-10 min. If seed moisture content is about 25.0% or more it should be pre-dried at 70°C for 2-5 hours, depending on the initial water content. The pre dried seeds should be kept in a closed desiccator for cooling. Then each of the duplicate quantities is weighed separately and about 20 g is ground. The ground material is then subjected to the moisture testing using an air oven as described below.

Moisture estimation

It should be carried out in duplicate on two independently drawn 5-10g working samples, weighed with an accuracy of 1 mg. Most species are dried for 1 hr at 130°C, cereals for 2 hrs (130°C) and maize for 4 hrs (130°C). Seeds containing high percentage of oil should be dried at 103°C for 17 hours.

**Steps:**

7. Empty container along with its cover should be weighed.
8. The submitted sample should be mixed thoroughly and two small portions of seed sample are weighed directly into the containers.

9. After weighing remove the cover or lid of the container and the open container should be kept in the oven which has already been heated to the prescribed drying temperature.
10. At the end of the drying period, container should be closed with its cover or lid.
11. The container should be transferred into a desiccator. The desiccator should be closed and the sample should be allowed to cool.
12. The sample should be weighed again and the moisture content may be calculated to one decimal place by the following formula.

$$m = \frac{m2 - m3}{m2 - m1} \times 100$$

Where m =seed moisture content.

m1 =weight of the empty container with its cover

m2= weight of the container with its cover and seeds before drying.

m3= weight of the container with its cover and seeds after drying.

The duplicate result of the determination may not differ by more than 0.2% otherwise the analysis should be repeated. If the material is pre-dried, the moisture content is calculated from the results obtained in the pre-dried and dried stages using the following formula.

$$M = S1 + S2 - \frac{S1 \times S2}{100}$$

M = moisture content

S1= moisture percentage lost in pre drying stage

S2= moisture percentage lost in drying stage.

**Moisture meters: universal Osaw digital moisture meters**

The principle involved in these moisture meters is that wet grains are good conductors while dry grains are less conductors of electricity. So the moisture content is directly proportional to the electrical conductivity of the seed.

It consists of a compression unit to compress the sample to pre-determined thickness. The thickness setting is very easily read on a vertical and circular scale. The seed material on test is taken in a test cup and is compressed. Then press the push type switch till the reading comes in the display. Here no temperature reading and correlated

dial are required. The computer versions of digital moisture meter automatically compensate for temperature corrections.

## 1.9 SEED HEALTH TESTING

It is done for determining the presence or absence of disease causing agents such as fungi, bacteria and viruses and insects in the seed samples. The pathogen may be carried with the seeds in the way.

**Admixture**

Pathogens are independent of seeds but accompany them. Ergot sclerotia are mixed with healthy seeds during threshing.

**External**

The pathogen may be present on seed surface as spores, oospores and chlamydospores as in case of karnal bunt of wheat, covered smut of barley, downy mildew of pearlmillet etc. By surface sterilization external seed borne diseases are killed.

Internal

Pathogens establish within the seed with definite relationship with seed parts.

**Procedure**

Working sample

The entire submitted sample, or a portion of it, depending on the test method may be used. Normally the working sample shall not be less than 400 pure seeds.

**Methods**

Examination without incubation

Such tests give no indication as to the viability of the pathogen.

Direct examination

The submitted sample, or a sub sample from it is examined, with or without a stereoscopic microscope and searched for ergots and other sclerotia, nematode galls, smut-balls, insects, mites and evidence of diseases and pests in seed or in inert matter.

**Examination of imbibed seeds**

The working sample is immersed in water or other liquid to make fruiting bodies, symptoms of pests etc., more easily visible, or to encourage the liberation of spores. After imbibition the seeds are examined either superficially or internally, preferably with the help of stereoscopic microscope.

Examination of organisms removed by washing

The working sample is immersed in water with a wetting agent or alcohol and shaken vigorously to remove fungal spores, hyphae, nematodes etc., intermingled with or adhering to the seeds. The excess liquid is then removed by filtration, centrifugation or evaporation and the extracted material examined with the help of a compound microscope.

Examination after incubation

After incubation for a specific period, the working sample is examined for the presence of symptoms of disease organisms, pests and evidence of physiological disturbances in the seeds and seedlings.

Blotters

These are used when pathogens are to be grown from the seeds or when seedlings are to be examined. The seeds with or without pretreatment are so spaced during incubation as to avoid secondary spread of organisms. Lighting is provided to stimulate sporulation of fungi when needed. Some pathogens can be identified without magnification but a stereoscopic microscope or a compound is often helpful in identifying spores.

Sand, artificial composts and similar media can be used for certain pathogens. The seeds usually without pre-treatment, are sown suitably spaced in the medium so as to avoid secondary spread of organisms and then incubated in conditions favourable for symptom expression.

In seed spices, Altenaria blight caused by Alternaria burnsii in cumin and Alternaria blight as well as Ramularia blight in fennel are reported to be a partial seed borne diseases. For the pathogenic test of these diseases, the following methods are used.

1.Standard blotter paper method:

400 seeds of each sample will be taken at random. Used sterilized blotter paper at the bottom of each petri dish aseptically and moisturized by sterile water. Ten seeds are placed at equal distance in each petri dish and incubate at 20-25$^0$C under room temperature. The petri dishes will be examined after 4-7 days of incubation for the presence of seed borne mycoflora and count the percentage of infected seeds.

Agar plates are used to obtain identifiable growth of organisms from seeds. Precautions should be taken to ensure their sterilization. The seeds, normally after pre-

treatment, are placed on the surface of sterilized agar and incubated. Characteristic colonies on the agar can be identified, either macroscopically or microscopically. Lighting is often useful and germination inhibitors may be used.

2. Agar plate Method

Four hundred seeds of each sample will be taken at random. Prepare PDA medium and ten seeds are placed at equal distance aseptically per petridishes containing 20 ml of PDA and incubate at 20-25$^0$c under room temperature.

The fungal growth on the seed will be examined regularlymand on seven days of incubation, count the percentage of infected seeds.Also transferred the individual fungal growth emerged from seeds on slant test tube by needle for pathogenecity. For sterilized condition, seeds will be treated with 0.1% $Hgcl_2$ solution for one minute followed by three washing of sterile distilled water, for unsterilized conditin, seed will be kept as such without any treatment. This method is more reliable than standard blotter paper method.

Examination of plants

Growing plants from seed and examining them for disease symptoms is sometimes the most practicable method for determining whether bacteria, fungi or viruses are present in the sample. Seeds from the sample under test may be sown or inoculum obtained from the sample may be used for infection tests with healthy seedlings or parts of plants. The plants must be protected from accidental infection from elsewhere and conditions may require careful control.

Other techniques

Specialized methods involving serological reactions, phage-plaque formation etc., have been developed for some disease organisms and may be used preferably in consultation with the seed pathologist.

Calculation and expression of results

Results are expressed as percentage by number of seeds affected or as number of organisms in the weight of sample examined. The result must be accompanied by statement of the test method used, including any pre-treatment applied, and of the amount of the sample or fraction examined. The absence of a statement concerning the health condition of the seed does not necessarily that the health condition is satisfactory.

Format of Seed Analysis Report

**MINI SEED TESTING LAB ANALYTICAL REGISTER (To be maintained by ADSCN)**

| Sl.No. | Name and address of the applicant | Crop/Variety | Class of the seed | Details of the seed source | Date of expiry | Quantity of the lot | Date of receipt | Date of sampling |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

| Date of test | Normal seedlings | Analytical result abnormal seedlings | Hard seed | Dead seed | Date of Despatch or result | File No. | Seed Rate recommended | Initial of ADSCN |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

**SEED SAMPLES AND SEED ANALYTICAL REGISTER (To be maintained by ADSCN)**

| Sl.No. | Name and address of the Producer | Kind/Variety | Class | S.C.No. | Lot No. | Quantity of the lot | Date of test |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| Purity | OCS | Analytical test results | | | Hard seed | Moisture | Remarks |
| | | WS | ODV/GP | Germination | | | |

| Name of the SCO | Date of drawing sample by SCO | Date of receipt of Sample by ADSCN | ADSC Code No. | Date of receipt of analytical report | Kind of sample Fresh Resample Validation | STL Code No. | Date of communication of result to the producer | Initials of ADSCN |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

**Seed labeling:** Seed of seed spices will be lebelled as per the tag and labels prescribed under the Seed Act 1964, as they are raised through seed.

**References:**


Malhotra, S.K. and Mehta, R.S. 2002. Seed certification standards for seed spices. Folder No. 1, pp 1-6.

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Parthasarthy, V.A. et al 2006. Detailed seed testing manual for spices crops. IISR, Calicut, 1-48.

Vari, A.K. et al 2004. Seed testing procedures and seed standards of sesbania and crotolaria. Division of Seed Science and Technology, IARI, N. Delhi, 1-20.

# PART 4 :
# PLANTATION CROPS

# *Seed and Planting Material Testing Manual*

# for

# Plantation Crops

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## Seed and Planting Material Testing Manual for Plantation Crops

**1. Name of the crop : Coconut**
**Botanical Name : *Cocos nucifera***

**Information regarding mother palms:**

**Mother palm selection**: The important features are: a) straight stout trunk with even growth and closely spaced leaf scars, b) spherical or semi-spherical crown with short fronds, c) short and stout bunch stalks without the tendency to drooping, d) more than 30 leaves and 12 inflorescences carried evenly on the crown, e) inflorescence with 25 or more female flowers, f) consistent yield of about 80 nuts, g) 150g per nut copra and h) absence of disease and pest incidence. Among all these characters, maximum attention is to be paid to the yield of nuts.

Regular and heavy bearers are selected as these are less affected by adverse weather conditions. In India, 80 nuts/palm/year is taken as the standard. High heritability values of 0.67 and 0.95 have been reported for yield of copra and weight of husked nut, respectively and as there is a significant correlation between these characters, weight of husked nut is taken as a reliable character for selection. It is also suggested that the palms which shows less loss in weight of embryo on self pollination should be selected because these are likely to have better breeding value.

Other factors to be considered are, size and shape of nuts and weight of bunches and nuts, as palms bearing medium sized, spherical/round nuts give higher copra and nut yield.

**VARIETIES :** The commonly grown varieties as well as released varieties/hybrids are listed below.

**1. TALL CUL TIV ARS**

These are most extensively grown in plantation scale in all coconut-growing tracts of the world. They are tall in stature growing to a height of 15 to 18m. Their life span extends from 60 to 100 years or more. They normally corne to bearing in about 6 to 10 years after planting and attain steady bearing in about 12 to 15 years. The nuts of the tall cultivars are generally medium to big in size with good quality and quantity of copra and fairly high in oil content.

Some of the popular tall cultivars are West Coast tall (WCT), East Coast Tall (ECT), Laccadive Ordinary Tall (LCT), Benaulim, Assam Tall, Andaman Ordinary Tall, Tiptur Tall, and Philippines Ordinary Tall (PHOT).

**2. DWARF CUL TIV ARS**

The dwarf cultivars are characterized by short stature and earliness in bearing. The palms commence bearing in about 3-4 years after planting. They are short-lived with a life span of about

40-50 years. The nuts are smaller, the copra is soft, leathery and oil content is low (66-68%). The dwarf cultivars exhibit three different nut colours namely, green, yellow and orange.

Some of the popular dwarf cultivars are Chowghat Orange Dwarf (COD), Chowghat Green Dwarf (CGD), Malayan Yellow Dwarf (MYD), Malayan Orange Dwarf (MOD), Malayan Green Dwarf (MGD) and Gangabondam (GBGD).

**RELEASED VARIETIES AND HYBRIDS OF COCONUT**

| Variety | Cultivar/ Parentage | State for which recommended |
|---|---|---|
| Chandra Kalpa | Laccadive Ordinary | Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra |
| Pratap | Banawali Green Round | Goa, Coastal Maharashtra |
| Kera Chandra | Philippines Ordinary | West Coast, Coastal Andhra Pradesh, West Bengal |
| VPM3 | Andaman Ordinary | Tamil Nadu |
| ALRI | Arasampatti Tall | Tamil Nadu |
| Kamrup | Assam Green Tall | Assam |
| Chowghat Orange Dwarf | Chowghat Orange Dwarf | Kerala, Tamil Nadu, Kamataka, Andhra Pradesh,Maharashtra |
| Chandra Sankara | COD x WCT | Kerala |
| Laksha Ganga (PHCl) | LCT x GBGD | Kerala |
| Chandra Laksha | LCT x COD | Kerala |
| Kera Ganga (PHC3) | WCT x GBGD | Kerala |
| Ananda Ganga (PHC2) | ADOTxGBGD | Kerala |
| Kera Sankara | WCT x COD | Kerala, Coastal Maharashtra, Coastal Andhra Pradesh |
| Kera Sree | WCTxMYD | Kerala |
| Kera Sowbhagya | WCTx SSAT | Kerala |
| VHC 1 | ECT x MGD | Tamil Nadu |
| VHC2 | ECT x MYD | Tamil Nadu |
| VHC-3 | ECT x MOD | Tamil Nadu |
| Godavari Ganga | ECT x GBGD | Andhra Pradesh |

**LOCATION:** Trees growing closer to households, cattle sheds, compost pits and other favourable conditions are to be avoided as it is not possible to differentiate inherently superior palms from those whose performance is due to favourable environment

**AGE:** Mother palms should be of 22 years old or more. Wherever possible, it is advisable to select middle-aged trees as they will be in their prime of life and it is easier to spot good yielder trom mediocre/poor yielder.

**METHOD OF SEED PRODUCTION:** ***OP/lnter se/Hybrid***
**SEED NUT SELECTION AND STORAGE:**

**a) Maturity of seed nut**

Generally, 10-12 month old mature nuts are used for seed purpose. The mature nuts should be harvested when at least one nut in the oldest bunch starts to dry. In Talls, it takes 11-12 months to become a mature seed nut whereas in dwarfs, nuts will mature in 10-11 months after pollination.

**b) Method of harvest**

Rope harvest of seed nuts is recommended trom the trees that are very tall and in places where the ground is hard.

**c) Selection of seed nuts**

Seed nuts are to be selected trom the center of the bunch, as development of nuts at top and bottom extremities may not be uniform leading to poor germination/poor quality of seedlings.

**d) Preservation of seed nut**

Harvested seed nuts are stored in shade to prevent drying of nut water. The seed nuts are generally stored till their husks become completely dry. Seed nuts of the tall variety can be stored for two months whereas the seed nuts of dwarfs should be sown with 15 days of harvest.

**GERMINATION PERCENTAGE**: The seeds can be sown in sand/potting mixture. Germination generally starts from the second month of sowing and continues till the sixth month of sowing, depending on the variety. Generally, germination is recorded till the fifth month of sowing and good seed lot will give more than 80% germination. However, in the case of Dwarf varieties such as the Chowghat Orange Dwarf a lower percentage of germination of around 65% is acceptable.

Seed nuts of tall palms will begin gennination within 90-130 days after sowing and 80-85% of seed nuts must have genninated within five months after sowing. Seed nuts of dwarf palms germinate 60-95 days after sowing.

**SEEDLING PRODUCTION:**

Method of seedling production: Nursery bed/ polybag

**Nursery site:**

The nursery site should preferably be in a level and well-drained area and should have loose or light textured soil. It should have a good source of water for irrigation, proper shade and be accessible for transportation. It should be far from potential sources of coconut insect pests and diseases.

**Seedbed preparation**

The seedbeds should be prepared in an area having loose and well-drained soil. The seed bed should have 1.5 m width, 10-20 cm height to provide drainage and of convenient length with 75 cm space between beds. The seedbeds should be drenched with Chlorpyriphos @ 0.05% before sowing of the seed nuts. To prevent bud rot/dry rot in the seedlings, the nursery can be drenched with Mancozeb @ 3%.

**Sowing of nuts**

The seed nuts are sown in rows with spacing of 30 cm (between rows) and 30 cm (between nuts) with four or five rows per bed. The nuts may be planted either horizontally with the widest of the segments at the top or vertically with stalk-end up. The nuts are the covered with soil, such that the top portion of husk alone is visible. Only seed nuts with nut water should be selected for sowing.

In areas having high incidence of insect pests and diseases, the seeds can also be treated with insecticides/pesticides prior to sowing. The seed nuts are soaked in water for one hour and then kept in a fungicidal solution (Mancozeb @ 3%) for two hours. In the case of insecticide treatment, the seed nuts are soaked in water for one hour and then kept in an insecticide solution (Chlorpyriphos @ 0.05%) for two hours.

A record file should be maintained indicating the name of variety sown, date of sowing, number of nuts sown, seedbed number and date of seed nut harvest. A signboard should be placed in front of each bed indicating the name of variety sown along with the date of sowing.

**Poly bag nursery:**

Poly bag nursery can be adopted for producing seedlings with greater vigour. As a result of the intensive care and better maintenance of the polybag nursery, vigourous seedlings with better root system are obtained, which attain rapid reproductive development. Compared to the nursery in the field, watering, weeding and culling operations for the elimination of unwanted seedlings are easier in poly bag nursery. The seeds can be sown in black polythene bags of 500-gauge thickness, 45 x 45 cm size for bigger nuts and 30 x 30 cm for smaller nuts. The bottom of the bags is to be provided with 8-10 holes for draining the excess water. To fill bigger bags around 10-12 kg and for smaller bags around 7-8 kg of potting mixture will be required. The commonly recommended potting media are top soil mixed with sand in 3:1 ratio or fertile top soil, sand or coir dust and well rotten and powdered cattle manure in the ratio of 3: 1 : 1. Red earth, well rotten and powdered cattle manure and sand in 1: 1 : 1 can also be used.

In order to produce poly bag seedlings, initially the seed nuts are sown very closely and

allowed to germinate in a pre nursery bed. The seed nuts start germinating about three months after sowing. The germinated nuts are picked out from nursery once a week till 80 % of nut germination or 5 months from sowing, whichever is earlier. The germinated nuts are then placed in the half filled bags with the sprout positioned upwards in the centre of the bag and sufficient potting mixture is added to fill the bags up to two-third portion and the sides slightly pressed to keep the nut firm. Usually the poly bag seedlings are maintained for about 8 to 10 months. The size of the poly bag nursery bed can be 6 x 3 m with about 115 cm spacing between beds for hose irrigation and other cultural operations.

**Seedling selection:**

Generally, seed nuts that do not germinate within 5 months after sowing as well as those with dead sprouts are removed from the nursery. Only good quality seedlings are retained in the nursery bed. Generally one-year-old seedlings are used for planting. Seedling selection is based on the following characteristics: (i) Six to eight leaves and (ii) Seedling collar girth of 10-12 cm. In addition, early splitting of leaves is a desirable characteristic. The petiole colour and vigour of the seedling are important traits for identification of the varietal status and hybrid seedlings. The root system should also be healthy, with at least 5-6 primary roots.

## 2. Seed and planting material testing manual for Cocoa

| Name of the crop | Cocoa |
|---|---|
| Botanical name | *Theobroma cacao L.* |
| Information regarding mother plants | Trees bearing 50-100 yellow pods/ year, smooth shallow furrowed fruits of 350 g, >35 beans of dry weight> 1.0 g should be selected. |
| Method of propagation | Seeds/ Budding/ Grafting |
| Seed propagation | Germination percentage- 90-95% in 10 days. Extract bold beans from middle of pod, keep seeds horizontally or vertically with hilum end down and just cover with sand. |
| Potting mixture | 2: 1: 1 soiI:FYM:sand, polybag 6"x9" sized 250 gauge. |
| Seed storage | cocoa seeds tend to lose viability and so sowing immediately after extraction is recommended. Over ripen pods showed viviparous germination also. Viability of the bean can be extended for some more days by storing freshly extracted seeds in moist charcoal and packed in poly bags. |
| Seed treatment | Rub with sand to remove mucilage. |
| Vegetative propagation | |
| Grafting/Budding | Soft wood grafting- Insertion of scion into the rootstock, tying tightly with poly strip of 1.5 cm width over joint and covering with poly pouch to avoid drying up of graft joint. 85% success in 2 months. |
| Scion preparation | |
| Age of scion shoots/buds | softwoods, thickness and physiological age same as root stock stem |
| Preparation of scion shoots/ buds | 12-15 cm long young shoots, defoliated, given with 'V' shaped slanting cut of 2-3 cm length (wedge) |
| Diameter of scion/size of bud | 1-1.5 cm diameter with 2-3 buds |
| Rootstock preparation | |
| Name of the rootstock | Bulk Forastero seeds |
| Age of the rootstock | 4 months old seedlings, decapitated, given with a vertical slit of2-3 cm down (cleft) |
| Diameter of the rootstock | 1-1.5 cm |
| Rootstock propagation | Seeds |
| Standards of the planting material | |
| Height of the plant | 60 cm |
| Height and condition of the union- | Compact union is at 30 cm height of the successful graft, polythene strip tied over grafts should be removed while planting and the graft |

| | |
|---|---|
| | joint should be above the soil in the pit. |
| Scion and root diameters at the union | 2 cm |
| Growth of the plant | straight with 5 to 6 pairs of leaves without branching |
| Root system of the plant | tap root branched |
| Condition of the earth ball | loose with equal spreading roots (before pot bound condition), transplant the grafts along with ball of earth |
| Disease incidence (Fungal, Bacterial, Viral etc) | Name of the disease- Seedling dieback/ blight |
| Causal organism | *Phytophthvra palmivora, Colletotrichum gloeosporioides* |
| Detection and diagnosis | Initiates from collar region, cotyledonary stalk or leaves as dark brown to black discoloration, spreads to the entire stem causing wilting, defoliation and ultimate death of the seedlings. Seedlings with leaf blight look very unhealthy, crinkling of leaves and stunted growth. |
| Treatment | Removal and destruction of infected seedlings from nursery to check the secondary spread of the disease. Incidence can be considerably reduced by improving the drainage facilities In the nursery and by providing proper shade. Drenching the seedlings with Bordeaux mixture (1%) or copper oxychloride (0.2%) just before the onset of monsoon and thereafter at frequent intervals will control the disease. |
| Insect pest incidence | Nil |
| Nematode incidence | Nil |
| Nutrient deficiency | Nil |

## 3. Seed and planting material testing manual for Arecanut

Arecanut palm *(Areca catechu* L.) is one of the important plantation crops of India. The crop occupies a prominent place among the cultivated crops in India especially in the states of Karnataka, Assam, Kerala, Maharashtra, Goa, Tamil Nadu, Meghalaya and Andaman & Nicobar group of Islands. India is the largest producer and consumer of arecanut and it continues to dominate world in area under cultivation and production and productivity.

The area under arecanut in India was 94,800 hectares during 1956-57, which increased to 3.72 lakh hectares during 2004-05. The production for the corresponding period has increased from 74,700 MT to 4.15 lakh in MT. Also productivity increased from 788 kg chali/ha in 1956-57 to 1186 kg chali per hectare during 2004-05. Thus the increase in production was not only due to increased area under cultivation but also increased productivity contributed by cultivation of superior varieties, supply of quality planting materials, better agro-techniques and plant protection measures.

Now, efforts are on to minimize the cultivation cost per unit area by introducing genetically superior high yielding varieties so that net return for the farmer is increased. Also due to yellow leaf disease and old and unthrifty plantations have resulted in decreased productivity which require replanting with elite planting materials. Hence, the production and productivity can be enhanced by planting quality materials of high yielding varieties of arecanut and also following the practices recommended for arecanut cultivation.

Systematic evaluation of exotic and indigenous accessions and selection for high yield and its component characters have resulted in release of high yielding varieties (Table I) and also identifying some of the promising cultivars (Table 2) for different agroclimatic conditions of the country.

**Table 1. Yield performance of released Arecanut varieties**

| Varietal name | Growth habit | Shape &size of nut | Yield chali/ kg/palm | State for which recommended | Year of release | Agency responsible for release |
|---|---|---|---|---|---|---|
| Mangala | Semi-tall | Round &medium | 3.00 | Coastal Karnataka and Kerala | 1972 | CPCRI |
| Sumangala | Tall | Oval& medium | 3.28 | Karnataka and Kerala | 1985 | CPCRI |
| Sreemangala | Tall | Round & bold | 3.18 | Kamataka and Kerala | 1985 | CPCRI |
| Mohitnagar | Tall | Oval to round & medium size | 3.67 | West Bengal, Coastal Kamataka and Kerala | 1991 | CPCRI |
| Calicut-17 | Tall | Elongated & bold | 4.37 | Andaman & Nicobar Islands | 1995 | CARI |
| SAS-1 | Tall | Round& medium | 4.60 | Valleys of Sirsi, Uttara Karnada, Karnataka | 1995 | RARS UAS Dharwad |
| Swarnamangala | Tall | Oblong to round | 3.78 | Kamataka and Kerala | 2007 | CPCRI |

**Table 2. Yield performance of Promising cultivars**

| Cultivar | Growth habit | Nut shape and size | Yield of chali/ kg/palm | Recommended agroclimatic zone |
|---|---|---|---|---|
| SK Local/ Kasaragod | Tall | round and bold | 2.00 | Northern Kerala and DK,Karnataka |
| Thirthahalli | Tall | small and elongated | 2.60 | Malnad areas of Karnataka |
| Sagar | Tall | small and round | 2.25 | Shimoga and Uttara Kannada of Karnataka |
| Shriwardhan | Semi-tall | round and medium sized | 2.00 | Coastal Maharashtra and Karnataka |
| Hirehalli local | Tall | round to oval & medium sized | 3.20 | Maidan Parts of Karnataka |

**Method of Propagation**

Arecanut is an exclusively seed propagated crop. Being a perennial and cross pollinated, it is essential that adequate care is taken in the selection of proper planting material.

**Method of seed production**

Open pollinated nuts can be collected from the compact blocks established by *inter se* material of respective varieties. *Inter* se mating is recommended in order to produce genetically superior pure planting materials and also to achieve greater homogeneity and allow completely random mating. Crossing is suggested to produce hybrids between desired parents. Selection of typical hybrid seedlings in the nursery is must.

**Mother palm selection**

The selected mother palm should be more than 12 years old with early bearing nature and with high fruit set. Palm should be regular bearer and consistent yielder. Presence of more number of leaves on crown is also a desirable trait and palms should be free from diseases and pest incidences.

**Seed propagation**

Fully ripened nuts having weight of above 35 g should be selected depending upon the varieties/cultivars. The nuts selected should float vertically with calyx-end pointing upwards when allowed to float on water. These nuts produce the seedlings of greater vigour. The germination should be more than 80 %.

**Method of seedling production**

**Primary nursery:** Selected seed nuts are sown 5cm apart in sand beds of 1.5m width and convenient length with their stalk ends pointing upwards. Thick mulching is to be done with straw. Beds are to be watered daily. The nuts usually commence germination by 43 days and complete by 94 days. The seedlings are retained in primary nursery for about six months.

**Secondary nursery:** For raising the seedlings in secondary nursery, beds of about 150cm width and 15 cm height is suitable. A spacing of 30-45 cm is considered optimum for planting three month old sprouts in secondary nursery. The secondary nursery should be given a basal dose of decomposed farmyard manure @ about 5 tonnes per ha. The sprouts from primary nursery can also be raised in polythene bags (25 x 15 cm, 150 gauge) filled with potting mixture (Top soil:FYM:sand=7:3:2). The nursery should be partially shaded for obtaining the quality seedlings.

Standards of the planting material

The seedlings selected should have maximum number of leaves (five or more) and minimum of 90 cm height with maximum girth at collar region. 12 to 18 month old seedlings are preferred for transplanting to obtain more vigorous palms with early flowering. The seedling should have well established root system with 5-8 main fibrous roots intact and active while transplanting.

**Diseases and pest incidences**

The major diseases noticed in the nursery or the transplanted seedlings are collar rot, leaf blight and leaf spot.

**Collar rot:** This is common in secondary nurseries and field planted seedlings. Infection by the pathogen is through collar region or root. Infection through collar region cause rotting of the growing bud while root infection leads to seedling wilt. Providing good drainage and soil drenching with I % Bordeaux mixture will reduce the incidence.

**Leaf blight:** This is due to different fungal pathogens. Reddish brown spots are formed on the leaf lamina. Severe disease leads to stunted growth of the seedling. Incidence of blight can be reduced by providing sufficient shade and spraying with any copper fungicide or dithane Z78.

**Leaf spot** : It is seen on 1- 1.5 year old seedlings. On young seedlings symptoms appear as yellow specks in summer. Severe infection cause stunted growth. Spots are brown to dark brown in colour with a yellow margin around. Severe infection cause drying, drooping and shedding of leaves.

Leaf spot seen on seedlings during summer can be reduced by providing proper shade and spraying with Dithane Z78 @ 4g/1 of water or 1 % Bordeaux mixture. Severely affected leaves may be removed and destroyed to avoid further spread of the disease. No major pest/insects and nematodes incidents seen in the seedlings.

# PART 5 :
# LABELS
# TO BE USED FOR
# HORTICULTURAL
# CROPS

**FORMULATION OF SIZE AND CONTENT OF LABELS TO BE USED FOR THE FRUITS, VEGETABLES, SPICES, PLANTATION CROPS, TUBER CROPS, , ORNAMENTAL AND FLOWER CROPS**

## 1. LABELS TO BE USED FOR VEGETATIVELY PROPAGATED FRUIT PLANTS

| **Name of the institution** | Tag No. |
|---|---|
| **Crop** | |
| **Variety** | |
| **Source of scion** | Nucleus/ Scion bank (Name) |
| **Clonal Class** | Grafted/budded /prepared by cutting/layering |
| **Name of the rootstock** | |
| **Type of rootstock** | Seedling/Clonal |
| **Batch No.** | |
| **Treatment details**<br>(Name of chemical and method) | Date of treatment |
| **Quality Testing** | Date of testing |
| **Freedom from diseases and pests** | Viral<br>fungal<br>bacterial<br>insect pests<br>nematodes<br>Others |
| **Date of packing** | |
| **Handling care** | |
| **Signature of Incharge with seal** | |

Disclaimer : The institution will not be responsible for any subsequent infections after sale of the material

Size of Label : For Breeder /Nucleus plants : 12X6 cm
For others : 15X7.5 cm
Color of Tags : Breeder plants – Clothlined Golden Yellow No. 356 (IS : 5-1978)
For others – Olive green

## 2. LABEL TO BE USED FOR SEED PROPAGATED CROPS

<table>
<tr><td colspan="2" align="center"> Tag No. Name of the institution</td></tr>
<tr><td>Crop</td><td></td></tr>
<tr><td>Variety</td><td></td></tr>
<tr><td>Class of seed</td><td>Breeder/Foundation/Certified/TL</td></tr>
<tr><td>Lot No.</td><td></td></tr>
<tr><td>Seed treatment (Name of chemical and method)</td><td>Date of treatment</td></tr>
<tr><td>Seed Testing</td><td>Germination (%)<br>Purity (%)<br>Date of testing</td></tr>
<tr><td>Weight when packed</td><td>Date of packing</td></tr>
<tr><td>Moisture level (%)</td><td></td></tr>
<tr><td>Signature of Incharge with seal</td><td></td></tr>
</table>

Disclaimer : 1. Valid for six months from the date of packing
2. Whether colored artificially-Yes /No

Size of Label : For Breeder seed/Nucleus seed : 12X6 cm
For others : 15X7.5 cm
Color of Tags : Breeder seed – Clothlined Golden Yellow No. 356 (IS : 5-1978)
For others – Olive green

### 3. LABELS FOR CROPS PROPAGATED BY DIVISIONS

| Tag No.<br>**Name of the institution** | |
|---|---|
| **Crop** | |
| **Variety** | **Lot No. :** **Weight :** |
| **Type of material** | Rhizome/tubers/corms/suckers/bulbs/cloves/offsets/runners |
| **Class of seed** | Nucleus/Breeder/Foundation/Certified/TL |
| **Treatment details** (Name of chemical and method) | Date of treatment |
| **Quality Testing** | **Sprouting (%)**<br>**Purity (%)**<br>Date of testing |
| **Freedom from diseases and pests** | Viral<br>fungal<br>bacterial<br>insect pests<br>nematodes<br>Others |
| **Date of Packing** | |
| **Signature of Incharge with seal** | |

**Disclaimer : The institution will not be responsible for any subsequent infections after sale of the material**

Size of Label : For Breeder seed/Nucleus seed : 12X6 cm
For others : 15X7.5 cm
Color of Tags : Breeder seed – Clothlined Golden Yellow No. 356 (IS : 5-1978)
For others – Olive green
For tissue culturally produced breeder seed : Diagonally divided Yellow and green

4. **LABELS TO BE USED FOR TISSUE CULTURALLY RAISED PLANTS**

<table>
<tr><td colspan="2" align="center">Tag No.<br>Name of the institution</td></tr>
<tr><td>Crop</td><td></td></tr>
<tr><td>Variety</td><td></td></tr>
<tr><td>Type of explant</td><td>Microplant/Microtuber/Microrhizome/Microcorm/Microbullb</td></tr>
<tr><td>Date of subculture/production</td><td></td></tr>
<tr><td>Batch No.</td><td></td></tr>
<tr><td>Treatment details<br>(Name of chemical and method)</td><td>Date of treatment</td></tr>
<tr><td>Quality Testing</td><td>Purity (%)<br>Date of testing</td></tr>
<tr><td>Freedom from diseases and pests</td><td>Viral<br>fungal<br>bacterial<br>insect pests<br>nematodes<br>others</td></tr>
<tr><td>Date of packing</td><td></td></tr>
<tr><td>Signature of Incharge with seal</td><td></td></tr>
</table>

**Disclaimer : The institution will not be responsible for any subsequent infections after sale of the material**

Size of Label : 15X7.5 cm

Color of Tags : Diagonally divided Yellow and green

5. **LABELS TO BE USED FOR ROOTSTOCKS**

<table>
<tr><td colspan="2" align="center">Tag No.<br>**Name of the institution**</td></tr>
<tr><td>**Crop**</td><td></td></tr>
<tr><td>**Name of the rootstock**</td><td></td></tr>
<tr><td>**Type of rootstock**</td><td>Seedling/Clonal</td></tr>
<tr><td>**Clonal class**</td><td>Prepared by cutting/layering/others</td></tr>
<tr><td>**Batch No.**</td><td></td></tr>
<tr><td>**Treatment details**<br>(Name of chemical and method)</td><td>Date of treatment</td></tr>
<tr><td>**Quality Testing**</td><td>Date of testing</td></tr>
<tr><td>**Freedom from diseases and pests**</td><td>Viral<br>fungal<br>bacterial<br>insect pests<br>nematodes<br>Others</td></tr>
<tr><td>**Date of packing**</td><td></td></tr>
<tr><td>**Handling care**</td><td></td></tr>
<tr><td>**Signature of Incharge with seal**</td><td></td></tr>
</table>

Disclaimer : The institution will not be responsible for any subsequent infections after sale of the material

Size of Label : For Clones : 12X6 cm
For others : 15X7.5 cm
Color of Tags : For clones – Clothlined Golden Yellow No. 356 (IS : 5-1978)
For others – Olive green

**6. LABELS TO BE USED FOR SEEDLINGS/SAPLINGS**

| Tag No.<br>**Name of the institution** | |
|---|---|
| **Crop** | |
| **Variety** | Wild /Indigenous/Name of the fruit species/variety |
| **Batch No.** | |
| **Treatment details**<br>(Name of chemical and method) | Date of treatment |
| **Quality Testing** | Date of testing |
| **Freedom from diseases and pests** | Viral<br>fungal<br>bacterial<br>insect pests<br>nematodes<br>Others |
| **Date of packing** | |
| **Handling care** | |
| **Signature of Incharge with seal** | |

**Disclaimer** : The institution will not be responsible for any subsequent infections after sale of the material

Size of Label : 15X7.5 cm
Color of Tags : Olive green

7. **LABELS TO BE USED FOR PLANTATION CROPS**

| Name of the crop | **Coconut** |
|---|---|
| Botanical Name | **Cocos nucifera** |
| Information regarding mother plants | |
| Variety | |
| Location | |
| Age | |
| Method of seed production | OP/Inter se/Hybrid |
| Seed Production details | |
| Date of seed harvest | |
| Seed storage | |
| Date of seed sowing | |
| Germination percentage | |
| Seedling Production Details | |
| Method of seedling production | Nursery bed/polybag |
| Age of seedling | |
| Seedling height | |
| Seedling collar girth | |
| Number of leaves | |
| Leaf splitting | Present/Absent |
| Seedling petiole colour | |
| Root system | |
| Disease incidence | Leaf spot/ /bud rot |
| Insect pest incidence | Scales/Mealy bug |
| Nature of damage | Low/moderate/Severe |

| Name of the crop | **Arecanut** |
|---|---|
| Botanical Name | **Areca catechu L.** |
| Information regarding mother plants | |
| Variety | |
| Location | |
| Age | |
| Method of Propagation | Seed |
| Method of seed production | Open Pollination/Inter se/Hybrid |
| Mother palm selection | |
| Age at first bearing | Early/Medium/Late |
| Bearing habit | Regular/Biennial |
| Fruit set | Low /medium/high |
| Germination percentage | |
| Method of seedling production | Nursery bed/polybag |
| Standards of the planting material | |
| Age of seedling | |
| Seedling height | |
| Seedling collar girth | |
| Leaf splitting | Present/Absent |
| Root system | |
| Disease incidence | Leaf spot/ Leaf blight/Collar rot |
| Insect pest/nematode incidence | Nil |
| Nature of damage | Low/moderate/Severe |