

Chapter - 6

METHODS OF PROPAGATION OF HORTICULTURAL CROPS

OBJECTIVES

After reading this chapter, the students will be able to:

- Enlist the advantages and disadvantages of sexual and asexual methods of propagation
- Explain the principles involved in sexual and asexual methods of propagation
- Demonstrate vegetative propagation methods used in the nursery
- Plan a business based on propagation & nursery management of horticultural plants

INTRODUCTION

Propagation i.e. multiplication is the law of nature. Every one, which comes in this universe produces individual of its own kind. Like animals, plants also multiply of their own by one or the other methods. However, man has developed several techniques of multiplication of plants for his own benefits. For propagation of plants, several principles are involved. In this chapter, attempts have been made to tell you about the principles of seed (sexual) and vegetative (asexual) methods of propagation. In addition, light will also be thrown on micropropagation.

Propagation of Fruit Plants

Propagation is an art and science of multiplication of plants. The fruit plants are propagated both by sexual and asexual methods. Most of the fruit plants are now propagated through grafting and budding, few through cuttings, layering, seeds and micropropagation. The propagation methods are broadly classified as sexual, asexual and micropropagation. The details of methods are presented below:

A. Sexual Propagation

Sexual propagation is the raising of plants by means of seed, which is formed by the fusion of male and female gametes within the ovule of a flower. Plants that

are produced from seeds are called seedlings. In ancient times when the asexual methods of plant propagation were not known this was the only commercial method for plant propagation. Papaya, phalsa and mangosteen are still being propagated by seed.

Advantages of sexual propagation

- Seedling trees are generally long-lived, bear more heavily and are comparatively more hardy.
- This is the only means of reproduction, where asexual propagation is not possible or economical, e.g., papaya, *phalsa*, mangosteen etc.
- To develop new varieties, hybrids are first raised from seeds and it is essential to employ this method in such cases.
- It has been responsible for the production of chance seedlings of superior attributes.
- Polyembryonic character exists in many fruit plants such as in some citrus species and some mango varieties and give rise to more than one seedling from one seed. The nucellar seedlings are true-to-type. Therefore, polyembryomic varieties can be propagated by seeds.
- Rootstocks upon which the fruit varieties are budded or grafted are mostly raised from seeds.
- Seedlings are cheaper and easy to raise.
- Seeds can be easily transported to distant places.
- Does not require high technical knowledge and skilled labour.

Limitations of sexual propagation

- Seedling trees are not uniform in their growth, yielding capacity and fruit quality.
- Seedling trees have long juvenile period and take more years to bear the first crop.
- Seedlings become large for economic management.
- It is not possible to maintain the exact character of any superior selection.
- Seed propagation can't be applied in many plants (e.g. banana).

- It is not possible to avail the modifying influence of rootstock on scion or scion on rootstock.
- Since seed-borne viruses exist in a number of fruit plants and the multiplication of such plants by seed is not recommended.

B. Asexual Propagation

Propagation of plants through any vegetative parts is called vegetative or asexual propagation. The goal of vegetative propagation is to reproduce progeny plants identical in genotypes to a single source plant.

Advantages of asexual propagation

- Vegetatively propagated fruit plants are true-to-type, uniform in growth, yielding capacity and fruit quality.
- Vegetatively propagated fruit plants come into bearing earlier.
- Uniformity in fruit quality makes harvesting and marketing easy.
- Modifying influence of rootstock on scion can be profitably availed off.
- It is possible to regulate the tree size, fruit quality, precocity etc., according to one's requirements by using different rootstocks.
- Cross pollination can be effected by grafting shoots of other suitable varieties (pollinizers) on some of the branches of self-unfruitful variety.
- Grafting can be used to encourage healing of tree wounds caused by rodents, implements, etc.
- Composite tree can be raised.
- One can correct (to some extent) the initial mistakes of planting inferior or unsuitable varieties.
- Fruit plants producing seedless fruits such as banana can only be multiplied through vegetative means.

Limitations of asexual propagation

- No new variety can be evolved by means of the vegetative method of propagation.
- It is more expensive than raising new plants through seeds.
- Vegetatively propagated plants are comparatively short-lived.

Propagation by Seed

In fruit plants, the propagation by seed is primarily done to raise rootstocks required for producing grafted or budded plants. However, some fruit plants (papaya) are conventionally propagated by seeds. Propagation through seeds requires a knowledge of several principles as described hereunder:

Dormancy : The dormancy in seeds may be due to hard seed coat, impermeability to water and gases, physiological immaturity of embryo, deficiency of some endogenous growth promoters or excess of endogenous growth inhibitors. Different methods like stratification, scarification and chemical treatment are used for breaking dormancy in seed to improve germination. Stratification consists of keeping seeds at low temperature (around 7°C) by placing them in alternate layers of moist sand. While, in scarification, the seeds are either treated with concentrated acid (acid scarification) or the seed coat is weakened mechanically, so as to make it permeable to water and gasses. In other instances, seeds can be pre-treated with some chemicals like thiourea or potassium nitrate to improve seed germination. Soaking seeds in water also overcome seed dormancy, if it is primarily due to endogenous growth inhibitors, which leaches due to prolonged soaking of seeds.

Germination : Germination refers to the emergence of a new plant from the mature seed. In other words, the activation of metabolic machinery of the embryo leading to the emergence of a new seedling plant is known as 'germination'. For the germination to be initiated, the seed must be viable, that is, the embryo must be alive and capable of germination and the environmental conditions must be appropriate. Seed germination involves different phases of germination. These phases are characterized by a rapid increase in water uptake during the imbibition phase followed by a period where there is little water uptake called the lag phase. During the third phase, there is increase in fresh weight of seed, which leads to the emergence of roots.

Apomixis : This is a natural mechanism during which vegetative embryo is produced instead of sexual or zygotic embryo. In other words, the apomixis occurs when an embryo is produced from a single cell of the saprophyte and does not develop from fertilization of two gametes. The seedlings produced are thus true-to-type and vigorous as compared to those produced through sexual means. Some species or individuals produce only apomictic embryos and are known as 'obligate apomicts', While others produce both apomictic and zygotic embryos are known as 'facultative apomicts', which occur in citrus.

Polyembryony : Polyembryony, as the name indicates, refers to the seeds having more than one embryo in the seed. One of the embryos arises from the union of

male and female gametes and is called gametic or sexual embryo, whereas the others are produced by simple mitotic division of cells of nucellus without the help of male gamete in their formation. The phenomenon of nucellar embryo is of common occurrence in citrus and mango.

In general, the nucellar seedlings are identified or in other words the zygotic seedlings are rouged out of nursery in step-by-step approach. In this method, the majority of the seedlings, which fall within one vigour group and are more or less of the same size, are considered to be nucellar. Others, which are either too small or too tall than the commonly prevailing type are discarded and considered to be off type or zygotic. To eliminate gametic seedlings, first rouging should be done when they are about 10 to 20 cm tall and ready for transplanting in the nursery. The second rouging should be done at time of budding, while third and final rouging should be done at time of selecting budded plants for transplanting in the field.

Vegetative Propagation

The propagation of plants by the method other than sexual propagation is referred as vegetative or asexual propagation. It involves no change in genetic makeup of the new plant. All the characteristics of the parent plant are reproduced in the daughter plant due to exact duplication of chromosomes during cell division. Thus, the plants are true-to type in growth, yield, ripening, and fruit quality.

Methods of vegetative propagation

There are different methods, which can be used for commercial multiplication of various fruit plants. These include cutting, layering, budding and grafting.

Cuttings

It is the method of propagating fruit plants in which the part of a plant (generally stem) having at least few buds, when detached from parent plant and placed under favourable conditions develop into a complete plant resembling in all characteristics to the parent from which it was taken. This method is commonly used in plants, which root easily and readily, thus, multiplication of plants is very quick and cheap. The fruit plants like phalsa, baramasi lemon and grapes are commercially propagated by cuttings.

The hardwood cuttings are the common method of propagation, which are prepared from fully mature tissues. Round cuttings are preferred over angular and immature



Hardwood cuttings

cuttings. The shoots of about one-year-old or more can easily be used for preparing hardwood cuttings. In case of deciduous fruit plants such as grape, pomegranate, *phalsa* and fig, the cuttings are made after pruning. While in evergreen fruit plants like baramasi lemon, the cuttings can be prepared during the spring (February - March) and rainy season (August-September). Generally, the cuttings of 15-20 cm length and having 3-5 buds are made. The lower cut is given in a slanting manner just below the bud to increase the absorption of nutrients. The upper cut is given at a right angle to reduce the size of the wound and as far as possible away from the upper bud to avoid its drying. After the cuttings are prepared, they should be allowed to dry. These cuttings are usually tied in small bundles (20-25 cuttings) and buried in moist soil/sand for a certain period for healing of wounds, which is known as callusing.

Layering

Layering is a method of vegetative propagation, in which roots are induced on the shoots while they are still attached to the mother plants. This is an alternate method of propagation in fruit plants which do not root easily when detached from the mother plants. Most commonly used methods of layering are air layering, ground and mound layering.

1. Air layering : As name refers, in this method the layering is done in air. To be more precise, the rooting is forced on the shoot itself when it is still attached to the mother plant. In this method, one-year old, healthy and straight shoot is selected and ring of bark measuring about 2.5 cm just below a bud is removed. Moist sphagnum moss is placed around this portion and is wrapped with a polythene strip. It should be light in weight, and should have a very high water holding capacity. If sphagnum moss is not available, any other material, which can retain moisture for long period, can be used for this purpose. The polythene covering does not allow the moisture to come out but permit gas exchange. Moreover, the layers need not be watered afterwards, which saves a considerable labour. This method of layering is also known as 'goottee' method.

Air layering can be practiced during February-March and July-August in guava, litchi, sapota, lemon, loquat etc. After few weeks, the roots are developed, which are visible through the polythene covering. Then a half way cut should be given to the rooted layers on the parent branch at least 15 days



Air layering



Rooting in apple stool plant

prior to their permanent removal from the mother plant. At the time of separation, a few leaves or small shoot is retained. It is also advisable to plant these rooted layers in nursery for close attention than to plant them directly in field. These layers can be planted in the fields during the following year in February-March or September-October.



Stooling in guava

2. Ground layering : In this method, a branch of plant, which is near the ground, is chosen and a ring of bark about 2.5 cm is diameter is removed just below the bud. This branch is then bended and buried in soil when still attached to the mother plant. The soil is regularly watered to keep it moist. Within a few weeks, the roots are formed and new plant is separated from the mother plant. Separation should be done in such a way that the roots formed also go with the detached plant. These new plants should preferably be planted in pots or nursery rows for development of better root system and shoot system before planting in the fields. This method is commonly followed for propagation of baramasi lemon.

3. Mound layering : In this method, plant is headed back either in February or in July. The new shoots come out during April and September, from ground level. A ring of bark is removed from these shoots and they are covered with moist soil. The rooted stools of April stooling are separated during rainy season and those of August are removed in the following spring. These stools, after separating from the parent plant are planted in the nursery fields. This method is also known as stool layering and is used for propagation of guava and apple rootstocks.

Budding

Budding is a method in which only one bud is inserted in the rootstock. This method is very easy and fast. This method saves budwood as compared to grafting. As soon as the bark starts slipping both on the stock and scion, this is considered to be the optimum time for budding. This shows that the cambium, which is the tissue responsible for union, is active. This method is generally employed during spring and rainy season. The common methods of budding are T-budding, patch budding, and chip budding.

1. T-Budding : This is also known as shield budding. A horizontal cut about 1/3rd the distance around the stock is given on the stock 15-20cm above then ground level. Another vertical cut, 2-3 cm in length is made down from the middle of the horizontal



T budding in citrus

cut and flaps of the bark are loosened with ivory end of the budding knife to receive the bud. After the 'T' out has been made in the stock, the bud is removed from the budstick. To remove the shield of bark containing the bud, a slicing cut is started at a point on the bud- stick about 1.25 cm below the bud, continuing underneath about 2.5 cm above the bud. A second horizontal cut is then made 1.25 to 2 cm above the bud, thus permitting the removal of the shield piece. The shield is removed along with a very thin slice of wood. The shield is then pushed under the two raised

flaps of bark until its upper horizontal cut matches the same cut on the stock. The shield should fix properly in place, well covered by the two flaps of bark, but the bud itself exposed. The bud union should be wrapped with polythene strip to hold the two components firmly together until the union is completed. 'T' budding can be performed at any time of the year provided cell sap flows freely.

In most fruit trees, it is performed either in the spring (March-April) or in rainy season (July-September) period. This is the most common method of propagation of citrus, *ber*, *aonla* plants.

2. Patch Budding : This type of budding is quite successful in guava and it gives 60 to 70 percent success during May and June. Freshly cut angular bud-wood from current season's growth should be used as scion. A rectangular or square patch or piece of bark about 1.0-1.5 cm broad and 2.5 cm long is removed from the rootstock at about 15 to 20 cm from ground level. A similar patch with a bud on it, is removed from the bud-stick taking care not to split the bark beneath the bud.

This patch is then transferred to rootstock and fixed smoothly at its new position and tied immediately with polythene strip. To have better success, a patch having two buds is used as scion instead of a single bud. This method is termed as improved patch budding method.

Points to remember

- Plants which shed their leaves in winter are easily propagated through hardwood cutting.
- Budding should always be performed when plants have active sap flow.
- Air layering (goottee) is highly successful if relative humidity is high.
- Epicotyl method of propagation is followed for mango in Konkan region of India where relative humidity is very high during monsoon season.
- Side veneer method of propagation is most successful for mango in which scion-sticks are forced to rest about a week

3. Chip Budding : This method is usually employed when the stock and scion are still dormant, that is just before the start of new growth. In this method, one about 2.5 cm long slanting cut is given into the stock followed by another cut at lower end of this first cut, in such a way that a chip of bark is removed from the stock. The bud from the scion wood is removed in the same way so that it matches the cuts given in the rootstock. This chip with a bud on it is fitted smoothly into the cut made in the rootstock taking care that the cambium layers of the stock and scion unite at least on one side. The bud is then tied and wrapped with polythene strip, to prevent drying up of the bud.

Grafting

Grafting is another method of vegetative propagation, where two plant parts are joined together in such a manner that they unite and continue their growth as one plant. In this method, the scion twig has more than two buds on it. Grafting is commonly done in pear, peach, plum, almond, mango etc. In temperate fruits like peach, plum and almond, grafting is done when the plants are dormant while, in mango, it is done when the trees are in active growth. The different methods of grafting are tongue grafting, cleft grafting, approach grafting, side grafting and veneer grafting.

1. Tongue Grafting : This method is commonly used when the stock and scion are of equal diameter. First, a long, smooth, slanting cut of about 4 to 5 cm long is made on the rootstock. Another downward cut is given starting approximately 1/3rd from the top and about 2 centimeter in length. Similar cuts are made in the scion wood exactly matching the cut given in the rootstock. The scion having 2 to 3 buds is then tightly fitted with the rootstock taking care that the cambium layer of at least one side of the stock and scion unites together. This is then wrapped tightly with polythene strip.



Approach grafting in mango

2. Cleft grafting : This is also known as wedge grafting. This method is useful in the nursery where the rootstock is quite thicker than scion and tongue grafting cannot be employed successfully. The stock up to 8 cm in thickness can be grafted with this method. The rootstock to be grafted is cut smoothly with a secateur or saw. It is then split in the middle down to about 4 cm. The budstick having 3 to 4 buds is trimmed like a wedge at the lower end with outer side slightly broader than the inner side. The lower bud on the scion should be located just well in to the stock

making sure that the cambium layers of both the stock and scion are perfectly matched. Cleft grafting is done during dormant period.

3. Approach Grafting : This method of grafting is termed as approach grafting, as the rootstock is approached to the scion, while it is still attached to the mother plant. Alternatively, the mother plants are trained to be low headed and the stock is sown under their canopy. Last week of July or the first week of August is the best period for approach grafting. In this method, the diameter of rootstock and scion should be approximately the same. A slice of bark along with a thin piece of wood about 4 cm long is removed from matching portions of both the stock and the scion. They are then brought together making sure that their cambium layers make contact at least on one side. These grafts are then tied firmly with a polythene strip or any other tying material. The stock and scion plants are watered regularly to hasten the union. The union is complete in about 2 to 3 months. The method is commonly followed in mango. This method is also called inarching.



Side grafting in mango

4. Side Grafting : A three sided rectangular cut about 4 x 1.25 cm is made on the rootstock at a height of about 15-20 cm from the ground level and the bark of the demarcated portion is lifted away from the rootstock. A matching cut is also made on the base of the scion to expose cambium. The scion should be prepared well before the actual grafting is done. The healthy scion shoots from the last mature flush are selected for this purpose. The selected scion shoots should have plump terminal buds. After the selection of the scion shoots, remove the leaf blades, leaving petioles intact. In about 7 to 10 days the petioles shall drop and terminal buds become swollen. At this stage the scion stick should be detached from the mother tree and grafted on the stock. The prepared scion is inserted under the bark flap of the rootstock so that the exposed cambia of the two components are in close contact with each other. The bark flap of the rootstock is resorted in its position. The graft union is then tied firmly with polythene strip.

After the completion of the grafting operation, a part of the top of rootstock is removed to encourage growth of the scion. When the scion has sprouted and its leaves turned green, the root stock portion above the graft union should be cut away. Side grafting can be carried out successfully from March to October; but success during the May and October is rather low. This method of propagation is commonly used in mango.

5. Veneer Grafting : In this method, a shallow downward cut of about 4 cm long is given on the rootstock at a height of about 15-20 cm from the ground level. At the base of this cut, a second short downward and inward cut is made to join the first cut, so as to remove a piece of wood and bark. The scion is prepared exactly as in side grafting. The cuts on the rootstock and scion shoot should be of the same length and width so that the cambial layers of both components match each other. Then, the prepared scion is inserted into the rootstock and tied securely with polythene strip. After the union is complete the stock is cut back, leaving time for doing veneer grafting.



Veneer grafting in mango

Propagation through specialized organs

1. Runners : A runner is a specialized stem that develops from the axil of a leaf at the crown of a plant. It grows horizontally along the ground and forms a new plant at one of the nodes (e.g. strawberry). The runner production is favoured by long day and high temperature. The daughter plants are separated and used as new planting material.



A view of runner production in strawberry

2. Suckers : A shoot arising on an old stem or underground part of the stem is known as suckers. In other words, a sucker is a shoot, which arises on a plant below the ground. These shoots, when separated from the mother plant and transplanted produce adventitious roots. The capacity of a plant to form suckers varies from plant to plant, variety to variety and is even climate dependent. The sucker formation is common in fruit plants like pear and banana. In banana, sword suckers are commonly used for propagation of plants.



Formation of suckers at the base of banana plant.

3. Slips : Slips are shoots just arising below the crown but above the ground. Pineapple is commercially propagated through this method of propagation.

Commercial methods of propagation of major fruits in India

Fruit crop	Commercial method of propagation	Fruit crop	Commercial method of propagation
Apple	Whip and tongue grafting, stooling	Grape	Hardwood stem cuttings
Peach	T budding	Grapefruit	T-budding
Plum	T budding	Guava	Stooling, Inarching
Pear	Tongue or whip grafting	Litchi	Air layering
Acid lime	Seed	Mandarin	T/shield budding
Avocado	Layering, T-budding	Mango	Inarching, Veneer grafting, Softwood grafting
Aonla	Patch budding	Pomegranate	Hardwood stem cuttings and Air layering
Bael	Patch budding	Pummelo	Seed, T-budding
Ber	Ring and T-budding	Sweet orange	T-budding
Custard apple	T-budding, Inarching, Offshoots	Date palm	Offshoots

PROPAGATION METHODS OF VEGETABLES

In India, a number of vegetable, plantation, medicinal and aromatic plants are grown commercially. In fact, we have a rich wealth of these plants in our country. These plants are raised from different propagation methods. Most annual vegetables are propagated by means of seeds. In some vegetables, the seeds are sown directly in the field, whereas in others, these are first sown in the nursery and then seedlings so produced are transplanted in the field afterwards. The time taken by the seed to germinate depends on various factors. In general, it takes 10 to 15 days for the seeds to germinate. The seedlings are planted in the field after about a month or nearly so.

The process of germination, factors affecting the germination process and other principles involved in seed propagation are given in detail in the previous chapters. Some vegetables are raised by vegetative methods also (Table 1).

Table 1: A list of vegetable crops raised by vegetative methods of propagation

Common name	Commercial propagation method	Common name	Commercial propagation method
Asparagus	Stem cuttings	Jerusalem artichoke	Sets of tubers with 2-3 eyes
Basella	Seeds; stem cuttings	Kakrol	Tuberous roots
Chow-chow	Whole fruit	Onion	Seeds and bulbs
Colocasia	Corm or cormel	Pointed gourd (Palwal)	Vine cuttings
Drumstick	Seeds and stem cuttings	Potato	Division of tubers; Seed potato weighing 40 to 50 g
Elephant's foot	Offsets of corms	Sweet potato	Vine cuttings with at least 4 nodes (about 40,000 to 50,000 cuttings are required /ha)
Garlic	Cloves and bulbils	Tapioca	Stem cuttings
Horse radish	Root cuttings	Yam	Division of tubers
Ivy gourd (Kundru or tondli)	Stem cuttings		

PROPAGATION METHODS OF FLORICULTURAL PLANTS

All plants having ornamental value are considered as the floricultural plants. These may be annuals, biennials, perennials, climbers, grasses, bulbous, herbaceous perennials, shrubs or trees. All these are propagated by seeds or some vegetative method of propagation (Table 2).

Ornamental bulbous plants

Many plants of horticultural importance are propagated by specialized structures like bulbs, corms, rhizomes and tubers. Bulbous plants are also commercially propagated from the above mentioned vegetative parts. Apart from these structures, these are

also propagated from other structures like offset, bulblet, cormlet, bulbils and scales etc (Table 3).

Table 2 : Propagation methods of some ornamental crops

Crop	Propagation method	Crop	Propagation method
Bougainvillea	Hardwood cuttings	Cacti and succulents	Seeds, cuttings of stem, leaf, bud cutting
Canna	Division of rhizomes	Carnation	Seed, terminal leaf cuttings
Chrysanthemum	Suckers and terminal cuttings	Climbers	layering or cuttage
Cycads and palms	Seeds, rarely by suckers and division of clumps	Dahlia	Leaf cuttings
Ferns	Spores	Flowering annuals	Seeds
Gerbera	Division of the mother plants	Gladiolus	Corms and cormlets
Herbaceous perennials	Seeds	Jasmine	Stem cuttings
Marigold	Seeds	Orchids	Seeds, tissue culture
Ornamental grasses:	Cuttings, stolon	Rose	T budding and cuttings
Tuberose	Bulbs		

Table 3. Major bulbous plants and their commercial propagation methods

Common name	Specialized structure used for propagation	Common name	Specialized structure used for propagation
Alocasia	Rhizomes	Corn lily	Corms
Amaryllis	Bulbs, bulblets	Crocus	Corms
Begonia	Tuberous roots	Crown Imperial	Bulbs
Belladonna lily	Bulbs and bulblets	Cyclamen	Tubers
Caladium	Tuberous rhizomes	Daffodil	Bulbs
Canna	Tuberous roots	Dahlia	Cuttings and tuberous roots
Gladiolus	Corms	Lily	Bulbs and offsets
Tuberose	Tubers	Tulip	Bulbs

Micropropagation

Propagation of plants under aseptic and controlled conditions of environment and nutrition under lab conditions is called as micropropagation. The vegetative propagation of plants has been practised for centuries and many improvements in conventional methods have been made over the years. Now, the tissue culture technique i.e., micropropagation has expanded their scope and potential on commercial scale. Micropropagation is suitable for the rapid and large-scale clonal multiplication of elite germplasm. The technique has been referred as micropropagation because the size of the tissue in culture is very minute as compared to conventional vegetative cutting or any other plant part. The meristem explant used for micropropagation is about 0.1-0.5 mm size having only one or two leaf primordia. With the advancement in science and technology, micropropagation technique has also been standardized for many plants, and it is now widely used for multiplication of many horticultural plants. The plant part to be cultured is called as explants, for which artificial culture medium is required.

Advantages of Micropropagation

Micropropagation has the following advantages over conventional propagation techniques:

1. Year around production of plants is possible.
2. Small space is required to maintain and multiply a large number of plants.
3. Small tissue is required as an explant, hence saves the scion wood to a great extent.
4. Speedy international exchange of germplasm, requiring minimum quarantine checks is possible.
5. Micropropagated plants exhibit vigorous growth, and higher yields.

Points to remember

- Propagation of plants through minute part under aseptic and controlled conditions of environment and nutrition under lab conditions is called as micropropagation.
- All micropropagation techniques are based on the phenomenon of totipotency of cell.
- It is a highly successful method of propagation in plants, which are also raised easily by vegetative means.
- Less field survival of tissue culture raised plants in the field is the main hindrance for expansion of this technique in India.

6. Micropropagated plants are usually free from viruses.
7. Micropropagation is highly beneficial in dioecious fruit plant species (date palm and papaya), where large-scale production of female plants is possible.
8. It helps in reducing the breeding cycle.
9. Production of homozygous plants is possible under *in vitro* conditions.
10. It is highly beneficial in plants in which vegetative propagation is not possible or the propagation rate is very slow (papaya and date palm).
11. *In vitro* systems have the potential for long-term transportation or shipment of propagation material.

Stages involved in micropropagation

There are four main stages involved in micropropagation of plants, such as explant establishment, shoot proliferation, rooting of shoots, hardening and transfer of plantlets to soil/field.

1) Explant establishment- The establishment of explant depends on several factors such as the source of explant/ genotype, type of explant such as leaf, root, stem from mature or immature plants/seedlings, explant sterilization, the *in vitro* culture conditions such as culture media, composition, temperature, humidity, light etc. The explants showing growth are considered established.



Proliferation of explant

2) Shoot multiplication- The established explants are subcultured after 2-3 weeks, on shoot multiplication medium. The medium is designed in such a way to avoid the formation of callus, which is undesirable for true-to-type multiplication of plants. Thus, careful use of auxins like NAA, 2,4-D and cytokinins like BAP, kinetin is done in culture medium. It is well-established fact that cytokinins enhance shoot multiplication.

3) Rooting of shoots- The *in vitro* regenerated shoots are rooted in the medium containing auxins like NAA, IBA. The rooting can also be induced when *in vitro* shoots are exposed to stress conditions. The rooting should also be preferably without formation of callus, thus avoiding somaclonal variants.

4) Hardening and transfer to soil/ field- The *in vitro* plantlets thus obtained are hardened/ acclimatized before transfer to the field. The hardening is necessary as the tissue culture derived plants grow under high humidity conditions, have open stomata, lower epicuticular wax, thus leading to increased transpiration losses and resulting in mortality of plants.



Rooted plantlets



Different steps involved in micropropagation of grape

Micropropagation Techniques

To produce virus free plants, meristem culture and micrografting techniques have been standardized in different fruit plants. The success varies with the plant species, variety and the culture environment. Different micropropagation techniques are discussed hereunder.

1. Meristem tip culture : This technique is widely used in horticultural plants like potato, dahlia, carnation and orchids. In this method, the meristem tip consisting of one or two pairs of leaf primordia are cultured in a medium. After a few weeks, the plantlets are re-generated and after hardening of the plantlets, these are transplanted in the soil under natural environmental conditions. Meristem tip cultured plants give rise to polyploid plants instead of diploid plants. Moreover, meristem tip culture is very useful for the elimination of viruses from infected plant material. Rapid multiplication of the plants, which are otherwise not easily propagated by vegetative means, is also possible through meristem culture. Plants produced are free from pathogens and can be stored for longer period and in smaller space.

2. Micrografting : It is difficult to regenerate complete plants from meristem in woody species like most of fruit and forest plants, thus as an alternative micrografting is done to produce virus-free plants. The various steps in micrografting include scion preparation, rootstock preparation, *in vitro* grafting and acclimatization/hardening of the plants. The *in vitro* raised nucellar seedlings are used as rootstocks. The scion (meristem 0.1-0.4 mm) is obtained either from young growth of field grown trees, defoliated glasshouse grown plants, or *in vitro* proliferated nodal segments obtained from mature trees. The grafting is done with the help of stereomicroscope, under aseptic conditions. Several viruses have been eliminated via micrografting in fruit plants such as Citrus Tristeza Virus, Peach latent mosaic viroid, Pear Vein Yellow virus.

Micropropagation techniques have been successfully adopted in many horticultural crops. Among fruit crops, strawberry was the first fruit to be propagated commercially through micropropagation. Micropropagation techniques have been standardized for many temperate, tropical and sub-tropical fruit crops. In India, tissue culture technique has been perfected in banana, grape and papaya. In banana, shoot tip excised from rhizomes of sword sucker are suitable explants and MS medium supplemented with sucrose (3 per cent), gelite (0.25 per cent), is the best. Shoot tips and two nodal microcuttings are highly suitable explants for faster and disease-free production of grape plants through tissue culture. Shoot tip culture technique has been demonstrated in papaya to produce female plants in the desired ratio.

Problems encountered during micropropagation

The success of micropropagation in several instances is hampered by the following problems:

1) Microbial contamination : Bacterial/fungal contaminations in the cultures do not allow the propagules to grow. This problem can be overcome by growing donor plants in growth chambers, systemic fungicide spray prior to explant removal, effective sterilization of explants, performing inoculations in laminar air flow cabinets fitted with HEPA filters (0.2 m) and using sterilized surgical instruments. Fumigation of inoculation room using dilute formaldehyde solution also helps to minimize this problem.

2) Browning of cultures : The cultured explants of certain plant species exude phenolic substances into the medium, which cause browning due to oxidation of phenols and formation of quinones, the toxins which effect the growth of cultured explants. The use of antioxidants such as activated charcoal (1-2%), citric acid or

ascorbic acid (50-100 mg/l) and polyvinylpyrrolidone (PVP), polyvinylpolypyrrolidone (PVPP) in the culture medium helps to check the browning.

3) Variability in tissue culture regenerated plants : Variability is highly undesirable in the micropropagated plants. It may occur due to callusing and regeneration of plants from callus instead of direct shoot induction and proliferation. Moreover, the plants regenerated through adventitious meristems as compared to axillary meristem are susceptible to mutations, as it is derived from either a single cell or a small group of cells. This leads to variation in regenerated plants. The variation due to callusing, can be overcome by addition of growth substances, such as, triiodobenzoic acid (TIBA), phloroglucinol and phloridzin which inhibit callusing and also by reduction of inorganic salt concentration in the culture medium.

4) Loss of plants due to transplantation shock : Tissue culture regenerated plants have anormal leaf morphology, poor photosynthetic efficiency, malfunctioning of stomata (open), reduced epicuticular waxes and thus are amenable to transplantation shock. Hardening of such plants is thus very essential before transplanting them under field conditions. Conservation of moisture by creating high humidity around the plants, partial defoliation and application of anti-transpirants are useful for hardening of *in vitro* raised plants.

Limitations of micropropagation

Micropropagation has certain limitations also. These are as under:

1. The facilities required are very costly.
2. Technical skill is required to carry out different micropropagation procedures.
3. Pathogens once appeared in the system, also multiply at a very faster rate in a short time.
4. Plants having high levels of phenols (mango, date palm, coconut etc.), usually do not respond to micropropagation techniques.
5. Establishment of laboratory-raised plants in the field is a very difficult task.

Hardening of micropropagated plants

The tissue culture plants need acclimatization or hardening before they are transferred in the field. The acclimatization is necessary because there is a vast variation in the environment surrounded by *in vitro* plants and the field environment. In culture vessels the *in vitro* plants are exposed to high humidity, heterotrophic mode of nutrition, high ethylene concentration and constant temperature throughout the year.

These conditions lead to the development of plants having low epicuticular wax, low stomatal density and stomatal malfunction, which make these plants more vulnerable to mortality in field conditions. To prevent this mortality, it is must to harden or acclimatize tissue culture plants.

Approaches for hardening of plants

To have success in hardening of tissue culture plants, the following approaches are adopted:

1. Balanced proportion of roots and shoots should be maintained in micropropagated plantlets.
2. Appropriate rooting media should be used for establishment of plants *ex vitro*.
3. Balanced nutrition should be provided for better survival of rooted plantlets.
4. Simultaneous rooting and acclimatization must be attempted.
5. Cleaning of gelling agents from roots before transfer to rooting media.
6. Moisture content or humidity around the transferred plantlets should be high.



ACTIVITIES/EXERCISES

- Go to a nearby fruit plant nursery. Make a list of plants being propagated there. With the help of the gardener (mali) find out the methods of propagation followed for the propagation of a particular fruit.
- Try to perform 'T' budding, air layering, side veneer grafting in horticultural plants propagated by these methods.
- Plan a visit a tissue culture laboratory of some ICAR institute or Agricultural University of your area. Make a list of instruments in that lab and try to learn about culture media, explants preparation, culture establishment etc.

CHECK YOUR PROGRESS

1. What are the advantages and disadvantages of vegetative method of propagation?
2. What is micropropagation? Discuss its advantages over other methods of propagation.
3. Why micropropagation technique of propagation could not be commercialized in several fruits and vegetables in India?

4. Write short notes on : T budding, air layering, hardwood cutting, side veneer grafting, and problems encountered during micropropagation.
5. Write propagation methods of vegetables and floricultural crops.

FILL IN THE BLANKS

- Citrus plants are primarily propagated by
- Grape is commercially propagated by
- Micropropagation protocols works on the principle of
- Micropropagated plants are free from
- Strawberry is propagated through.....
- Banana is propagated by.....
- Scarification is done to
- Stratification treatment is given to
- In micro-grafting, the size of explants is
- Papaya is mainly propagated by

SUGGESTED FURTHER READINGS

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