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Micro Propagation: A Special Technique of Propagation in Horticultural Crops

Anshul S. Lohakare
Assistant Professor, College of Horticulture, V.N.M K.V. Parbhani (M.S.)

SUMMARY

Apart from conventional methods of propagation such as sexual and asexual methods, there is a special technique of propagation, which has played an important role in enhancing efficiency of plant production. One of such methods is in vitro, also commonly referred as micro propagation.

INTRODUCTION

The technique has been referred as micro propagation 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 micro propagation is about 0.1-0.5 mm size having only one or two leaf primordia. Morel and Martin (1952) for the first time demonstrated that virus free plants can be obtained by culturing shoot meristems. Later on with the discovery of the hormonal control of organogenesis by Skoog and Miller (1957) and finding of most commonly used T/C media by Murashigue and Skoog (1962), the scope of micro propagation was further extended to vast range of plant species, including fruit and plantation crops. With the advancement in science and technology, micro propagation technique has also been standardized for many plants, and it, is now widely used for multiplication of many horticultural crops.

Fig. 1: Micro Propagation Steps in Banana

Stages involved in Micro propagation- There are four main stages involved in micro propagation of plants as mentioned below.

1) Explant establishment- The establishment of explant depends on several factors such as the source of explants / 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.

2) Shoot multiplication- The established explants are subculture 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 the 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.

Micro propagation Techniques

To produce virus free plants, meristem culture and micro grafting techniques have been standardized in different fruit plants. The success varies with the plant species, variety and the culture environment.

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. Micro grafting: It is difficult to regenerate complete plants from meristem in woody species like most of fruit and forest plants, thus as an alternative micro grafting is done to produce virus free plants. The various steps in micro grafting include scion preparation, rootstock preparation, *in vitro* grafting and acclimatization/ hardening of the plants. The *in vitro* raised nuclear seedlings are used as rootstocks. The scion (meristem 0.1-0.4 mm) is obtained either from young growth of field grown trees 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* micro grafting in fruit plants such as *Citrus tristeza virus*, *Peach latent mosaic viroid* and *Pear yellow vein virus*.

Micro propagation techniques have been successfully adopted in many horticultural crops. Among fruit crops, strawberry was the first fruit to be propagated commercially through micro propagation. In India, tissue culture technique has been perfected in banana, grape and papaya. As in banana, shoot tip excised from rhizomes of sword sucker are suitable explants and MS medium is the best. Shoot tips and two nodal micro cuttings 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. Despite the success in micro propagation in several instances is hampered by encountering many problems which are given as below.

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, spray of systemic fungicide prior to explant removal, effective sterilization of explants, performing inoculations in laminar air flow cabinets fitted with HEPA filters (0.2 µm) 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 secrete phenolic substances into the medium, which cause browning due to oxidation of phenols and formation of quinones, the toxins which affect 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 T/C regenerated plants: Variability is highly undesirable in the micro propagated 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. Thus leads to variation in regenerated plants. The variation due to callusing, can be overcome by addition of growth substances which inhibit callusing such as Tri-Iodobenzoic Acid (TIBA), phloroglucinol and phloridzin 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 a normal 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 must before transplantation under field conditions.

Hardening of Plants:

The tissue culture plants need acclimatization or hardening before they are transferred in the field. The acclimatization is necessary because there is 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 must to harden or acclimatize tissue culture plants. To have success in hardening of tissue culture plants, the following approaches are adopted:

1. Balanced proportion of roots and shoots in micro propagated plantlets.
2. Appropriate rooting media for establishment of plants *ex vitro*.
3. Balanced nutrition for survival of rooted plantlets.
4. Simultaneous rooting and acclimatization.
5. Cleaning of gelling agents from roots before transfer to rooting media.
6. Moisture content or humidity around transferred plantlets.
7. Plantlets should be hardened in mist/greenhouses prior exposing them to open conditions.

Advanced Approaches: An alternative *in vitro* and *ex vitro* approaches can be adopted.

1. Pre hardening of plantlets in culture vessel, before transfer to soil.
2. Alterations in sugar concentration in the culture medium.
3. Concentration of gelling agents.
4. Use of anti transpirants.
5. Control of gas exchange around the plantlets.
6. Use of growth retardants.
7. Autotrophic mode of nutrition of *in vitro* plantlets.

Fig. 2: Hardening of tissue culture plantlets under greenhouse.

Advantages of Micro propagation:

- Year round production of plants irrespective of seasonal constraints.
- Small space is required to maintain and multiply large number of plants.
- Small tissue is required as an explant, hence saves the scion wood to a great extent.
- Speedy international exchange of germplasm, minimum quarantine checks is possible.
- Micro propagated plants exhibit generally vigorous growth and higher yields.
- Micro propagated plants are usually free from viruses.
- Micro propagation is highly beneficial in dioecious fruit crops.
- It helps in reducing the breeding cycle.
- Production of homozygous plants is possible under *in vitro* conditions.
- It is highly beneficial in plants where vegetative propagation is not possible.
- Micro propagation can be done in plants where vegetative propagation rate is very slow.
- Long-term transportation or shipment of propagation material is possible in *n vitro* systems.

Disadvantages of Micro propagation:

- The facilities required are very expensive.
- Technical skill is required to carry out different micro propagation procedures.
- High risk of contamination.
- Plants having high levels of phenols (Mango, Date Palm, Coconut etc.), usually do not
- respond to micro propagation techniques.
- Establishment of laboratory-raised plants in the field is a very difficult task.
- Cost of cultivation increases due to the cost of tissue culture plantlets.

CONCLUSIONS

The vegetative propagation of plants has been practiced for centuries and many improvements in conventional methods have been made over the years. Recently, the tissue culture technique i.e. micro propagation has expanded their scope and potential on commercial scale. Micro propagation is suitable for the rapid and large-scale clonal multiplication of elite germplasm. This technique has been standardized for many temperate, tropical and sub-tropical fruit crops.

REFERENCES

Aldriance G.W. and Brison F.R. (2000) Propagation of horticultural plants. Mc Grow Hill Book Company. Inc, New York.

Bose T.K., Mitra S.K., Sadhu M.K. and Das P. (1997) Propagation of Tropical and Sub tropical Horticultural Crops. IInd Edition, Naya Prakash, Calcutta.

Morel G. and Marthin C. (1952) Guerison de Dahlias attaints d'une maladie a. virus. CR Hebd Séances Acad, 253, 1324-1325.

Murashige T. and Skoog F. (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol Plantarum*, 15, 473-497.

Sharma R.R. (2002) Propagation of Horticultural crops: Principles and Practices. Kalyani Publishers, New Delhi.

Skoog F. and Miller C.O. (1957) Chemical regulation of growth and organ formation in plant tissue cultured *In vitro*. *Symp. Society for Experimental Biol*, 11, 118-131.