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Micropropagation in Plant Tissue Culture

Gorantla Nagamani

Research scholar, Department of molecular Biology and Biotechnology, PJTSAU, Rajendranagar, Hyderabad
E-mail: nagamanigorantla23@gmail.com

Multiplication of genetically identical copies of a cultivar by asexual reproduction is called Clonal propagation. In nature, clonal propagation occurs by apomixis or vegetative propagation. Tissue culture has become popular method for vegetative propagation of plants. Aseptic method of clonal propagation is called as Micropropagation and it offer the advantage of large number of true-to-type plantlets can be produced with relatively short time and space from a single individual. It is the fact that micropropagation is the only commercially viable method of clonal propagation of most of the horticultural crops. E.g. Orchids (Gracia-Gonzales, 2010).

Stages in Micropropagation:

Micropropagation generally involves five stages. Each stage has its own requirements (Sagare *et al*., 2010).

Stage 0: Preparative stage

This stage involves the preparation of mother plants to provide quality explants for better establishment of aseptic cultures. To reduce the contamination problem in the subsequent stages, mother plant should be grown in a glasshouse and watered so as to avoid overhead irrigation. This Stage also includes exposing the stock plants to suitable light, temperature and growth regulator treatments to improve the quality of explants. In the case of photosensitive plants it may be possible to obtain suitable explants throughout the year by controlling photoperiod in the glasshouse.

Stage 1. Initiation of culture

Explant: The nature of explant to be used for *in vitro* propagation is governed by the method of shoot multiplication. For enhanced axillary branching, only the explants which carry a pre-formed vegetative bud are suitable. When the objective is to produce virus-free plants from an infected individual it becomes necessary to start with sub-millimeter shoot tips. If the stock is virus-tested or virus eradication is not necessary, then the most suitable explant is nodal cuttings. **Sterilization:** Special precautions need to be taken when explants are derived from field grown materials, In such cases an ideal approach would be to take cuttings from the selected plant and grow them in greenhouse. Discarding the surface tissues from plant materials while preparing the explants also minimizes the loss of cultures due to microbial contamination

Stage2. Multiplication: This is the most crucial stage in micro propagation. Broadly three approaches have been followed to achieve *in vitro* multiplication.

A. Through callusing

B. Adventitious bud formation

C. Enhancing axillary branching

A. **Through callusing:** The potentiality of plant cells to multiply indefinitely in cultures and their totipotent nature permit a very rapid multiplication of several plant types. Differentiation of plants

from cultured cells may occur via organogenesis or somatic embryogenesis. Somatic embryogenesis is most appealing from a commercial angle. Since somatic embryos are bipolar structures, with defined root and shoot meristems, the rooting stage required for microshoots gets eliminated. Above all, somatic embryos being small, uniform and bipolar are more amenable to automation at the multiplication stage and for field planting as synthetic seeds. it can be stored through cold storage, cryopreservation or desiccation for prolonged periods. The most serious objection against the use of callus cultures for shoot multiplication is the genetic instability of their cells.

B. Adventitious bud formation: Buds arising from any place other than leaf axil or the shoot apex is termed adventitious buds. The shoots differentiated from calli should also be treated as adventitious buds. For most bulbous plants (e.g. Lilley) adventitious bud formation is the most important mode of multiplication and the best explants are obtained from bulb scales.

C. Enhanced axillary branching: In cultures the rate of shoot multiplication by enhanced axillary branching can be substantially enhanced by growing shoots in a medium containing a suitable cytokinin at an appropriate concentration with or without auxin. Due to continuous availability of cytokinin, the shoots formed by the bud, present on the explant, develops axillary buds which may grow directly into shoots. This process may be repeated several times and the initial explant transformed into a mass of branches.

Stage 3 Rooting of shoots.

Somatic embryos carry a pre-formed radical and may develop directly into plantlet. Adventitious and axillary shoots developed in cultures in the presence of a cytokinin generally lack roots. To obtain full plants the shoots must be transferred to a rooting medium which is different from the shoot multiplication medium, particularly in its hormonal and salt compositions. For rooting, individual shoots are excised and transferred to the rooting medium containing auxin source (Hussain *et al*., 2012).

Stage 4 Transplantation

The ultimate success of commercial propagation depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rates. The two main deficiencies of *in vitro* grown plants are – poor control of water loss and heterotrophic mode of nutrition. Therefore, gradual acclimatization is necessary for these plants to survive transition from culture to the greenhouse or field. During acclimatization the *invitro* formed leaves do not recover but the plant develops normal leaves and functional roots. While transferring out shoots/roots their lower part is gently washed to remove the medium sticking to them. The individual shoots or plantlets are then transferred to potting mix and irrigated with low concentration of inorganic nutrients. This probably recommissions the photosynthetic machinery of plants, enabling them to withstand the subsequent reduction in the ambient relative humidity and survive under field conditions. A variety of potting mixtures such as peat, perlite, polystyrene beads, vermiculate, fine bark, coarse sand etc. or their mixtures in different combinations are used for transplantation. For initial 10-15 days, it is essential to maintain high humidity (90-100%) around the plants, to which they got adapted during culture. The humidity is gradually reduced to ambient level over a period of 2-4 weeks (Hussain *et al*., 2012).

Advantages

- Clonal mass propagation - extremely large numbers of plants can be produced.
- Culture is initialized from small parts of plants – less space is required: from 1 m2 space in culture room, 20000 - 100000 plants can be produced per year.
- Production of disease and virus free plantlets.
- Increase the production of plants that normally propagate very slowly such as bulbous crops.

- Introduction of disease free new cultivars is possible through micropropagation
- Vegetative propagation of sterile hybrids can be used as parent plants for seed production. E.g. Cabbage
- One of the rapid methods for cloning of disease free trees.
- In vitro cultures can be stored for long time through cryopreservation.
- Breeding cycle can be shortened (El-Dougdoug and El-Shamy, 2011).

Disadvantages

- Expensive laboratory equipment and service.
- Plants are not autotrophic
- Poor Acclimatization to the field is a common problem (hyperhydricity)
- Risk of genetic changes if 'de novo' regeneration is used
- Mass propagation cannot be done with all crops to date. In cereals much less success is achieved
- Regeneration is often not possible, especially with adult woody plant material.
- More problems in inducing rooting.
- May not get uniform growth of original plant from tissue culture. Each explant has different in vitro growth rates and maturation. Thus cannot be used for floriculture crop production where uniformity is critical (Hussain *et al*., 2012).

References

El-Dougdoug KA, El-Shamy MM (2011) Management of viral diseases in banana using certified and virus tested plant material. African Journal of Microbiology Research. 5(32): 5923-5932.

Garcia-Gonzales R, Quiroz K, Carrasco B, Caligari P (2010) Plant tissue culture: Current status, opportunities and challenges. Ciencia e Investigación Agraria. 37(3): 5-30.

Hussain, A., Ahmed, I., Nazir, H., & Ullah, I. (2012). Plant Tissue Culture: Current Status and Opportunities. Recent Advances in Plant in Vitro Culture.

Sagare AP, Lee YL, Lin TC, Chen CC, Tsay HS (2000) Cytokinin-induced somatic embryogenesis and plant regeneration in *Coryodalis yanhusuo* (Fumariaceae)- a medicinal plant. Plant science. 160: 139-147.