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Micropropagation of Plants-Stages and Applications

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Micropropagation is a method of propagating plant using very small parts of plants that are grown under aseptic conditions in a balanced diet of chemicals. Micropropagation is a sophisticated technique for the rapid multiplication of plants and as such several million plants could be produced from one shoot tip in a single year. The process is season independent and ensures plant production round the year. *In vitro* Micrografting supports micropropagation of a broader host range of plant species, especially for the multiplication of woody plants and hardwood perennials (Wang et al., 2022)

The concept of micropropagation was first presented to the scientific community in 1960 by the Morel producing virus – free cymbidiums through the technique of meristem culture. Today the technique developed by Morel is used worldwide and in few commercially important genera such as *Phalaenopsis* and *Paphiopedilium* (orchids) which once remained fairly not feasible to micropropagate, are now propagated. However, credit for the development of the field of micropropagation is also shared by Murashige who showed that many plants, in addition to Orchid could be propagated *in vitro*.

Micropropagation consists of three types of vegetative propagation

1. Axillary shoot production where axillary buds and meristems give rise to shoots that are excised and used to produced additional such shoots.
2. Adventitious shoot production, comprising *do novo* meristem formation form callus tissue or directly from organized tissues such as epidermal or sub epidermal cells
3. Somatic embryogenesis in which structures are formed containing a shoot and root connected by a close vascular system (directly analogous to zygotic embryos are regards to histology, physiology and biochemistry)

Among these three types of vegetative propagation for micropropagation, first two types are widely applicable in most of the commercially important plant species. Micropropagation through somatic embryogenesis is limited to very few crops.

Stages of Micropropagation

Micropropagation is divided into 5 stages. 1-4 were originally proposed by Murashige; Debergh and Maene added stage O.

State 0: Preparative stage: Donor plant selection and preparation.

Explant quality and responsiveness is influenced by the physiological and phytosanitary condition of the donor plants.

- Donor plants must be maintained in clean conditions under low humidity to reduce pathogen infections
- Donor plant should be indexed for the pathogens.
- Vigorously growing donor plants must be selected.
- Donor plants may be pretreated to avoid fungal/bacterial/nematode infections.

Stage 1: Establishment of explant in culture

- Surface sterilization: The explants are sterilized with surface steriliants such as sodium hypochlorite, mercuric chloride, 70% ethanol, bromine water, silver nitrate etc., which are not phytotoxic.
- Medium composition: The medium should provide the explants with all nutrients that are necessary to nourish and to make the explant grow as desired by adjusting the concentration of plant growth regulators. Medium formulation is often standard, e.g. M.S but more complex media may be necessary for smaller explants.
- Controlled conditions: The explants inoculated in the media should be maintained under optimum light (1 Klux to 10 Klux), temperature (25 ± 2^0C) and relative humidity (>75%). Under these conditions the culture will be established. The time required for stabilization may be negligible or very long which depends on the genotype and explant. Eventually, the culture stabilizes, and then it adapts to culture and begins to grow steadily.

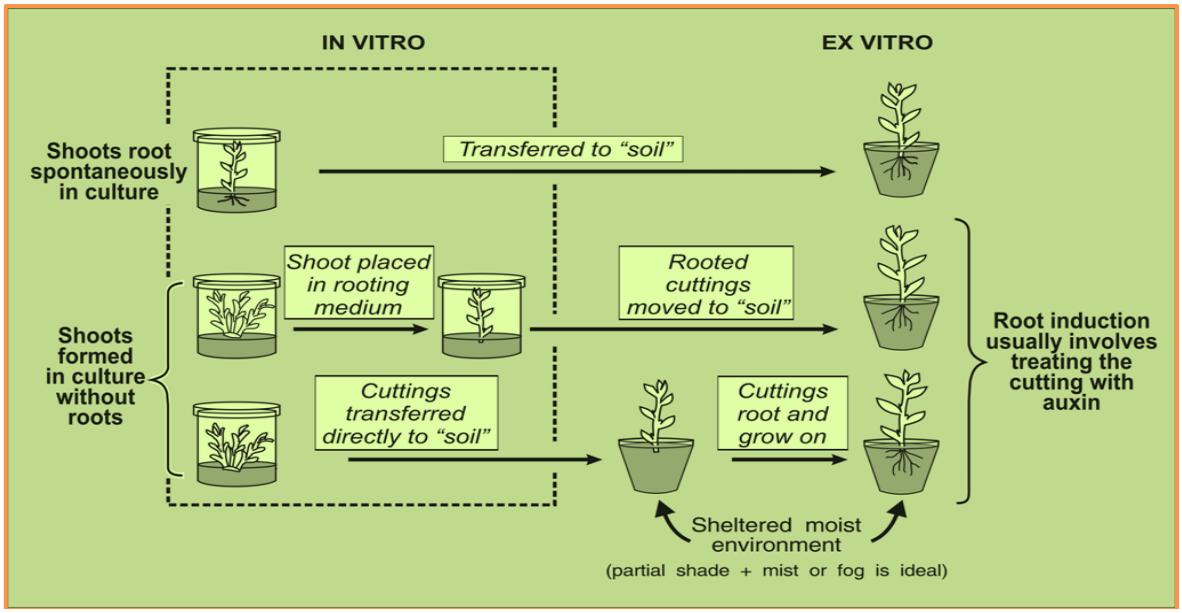
Stage 2: Multiplication: Proliferation of axillary shoots

- Repeated enhanced axillary shoot production is encouraged by cytokinin in the medium, alone or with a smaller amount of auxin. Cytokinin promotes bud growth and several shoots grow for every shoot when placed in culture.
- Every four to eight weeks, the shoots are divided and placed on fresh medium to repeat the process. Surplus shoots are channeled into propagation.
- Number of subcultures possible from the original culture varies with species/cultivar. More numbers of subcultures leads to reduced growth, increase in mutations and somaclonal variation.

Stage 3: Pretransplant (Rooting)

- Harvested shoots may be pretreated before rooting. Pretreatment may include elongation and prehardening.
- Single shoots and shoot clusters are treated with auxin, usually either by putting the shoots on auxin containing medium or by dipping them in auxin solution and planting on hormone – free medium. Rooting by transferring the shoots to auxin containing medium is widely followed.

Rooting shoots is a very important part of any *in vitro* propagation scheme. A few species form adventitious roots on shoots during the course of Stage III culture, but usually it is necessary to adopt a separate rooting procedure using special media, or methods, to induce roots to form. Sometimes shoots may need to be specially elongated before rooting is attempted. To reduce the costs of micropropagation, many laboratories now remove unrooted shoots from the *in vitro* environment and root them outside the culture vessel.



Alternative methods of rooting micropropagated shoots

Stage 4: Acclimatization

- It is a process by which physiologically and anatomically plantlets adjust from *in vitro* to *ex vitro* conditions.
- Rooted shoots are transferred into clean potting medium and grown under mist. Acclimatization is relative a slow process and may take weeks. The plantlets should possess enough starch reserves for its metabolic activities during acclimatization.
- The plantlets must adjust to low RH (20-60%) by developing sufficient defense such as epicuticular wax deposition to arrest water losses
- The plantlets much adjust from low light to high light from low photosynthesis competence by independently carbon fixation through proper development of chloroplasts.
- Acclimatization structures such as humidity temperature, overhead mist, fog system and plastic covers must be maintained.

Advantages of micropropagation

- Rapid multiplication of desirable plants.
- Production of pathogen – free plants
- Tissue culture plant are available throughout the year
- Rapid dissemination of plants all-round the year throughout the world
- Export / Import of tissue culture plant are easier as it occupies less space.
- Germplasm storage.

Disadvantages

- High production costs
- High labour input
- Loss of cultures/plantlets due to contamination
- Loss of plantlets due to low survival in green house
- Variations in induced due to *in vitro* conditions.

Applications

- Clonal mass propagation of large number of disease free uniform plants
- Micropropagation may allow faster production of plants that are slow to propagate *in vivo*.
- It may decrease the time needed for bulk of new cultivars before they are introduced commercially
- Storage of germplasm, e.g. by cryopreservation.

In recent years, usage of bioreactors for plant propagation has increased the production of healthy plants under aseptic conditions. Temporary immersion, and other modified bioreactors have been largely used for tissue culture plant production. Bioreactors are used for the regeneration of propagules, like shoots, bulblets, microtubers, cormlets and rhizomes (Murthy et al., 2023).

References

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