MBT 303

Micropropagation

What is micropropagation?

Growing any part of the plant (explants) like, cells, tissues and organs, in an artificial medium under controlled conditions (aseptic conditions) for obtaining large scale plant propagation is called micropropagation.

Micropropagation generally involve following stages:

1. Stage 0: Preparative stage
   Involves is the selection of mother plant and preparation of explant

2. Stage 1: Initiation of culture
   (a) Explant
   (b) Culture medium
   (c) Sterilization
   (d) Inoculation and incubation

3. Stage 2: Multiplication
   (a) Through callusing
   (b) Adventitious bud formation
   (c) Axillary bud multiplication

4. Stage 3: Rooting of shoots

5. Stage 4: Transplantation
   This stage usually involves a hardening-off process and acclimatization of plants in soil under green-house conditions for later transplanting to the field.

Hardening:

Involve change from Heterotrophic to Autotrophic

Method

Plantlets → **Take out from culture medium**

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Wash carefully to remove any Medium attached on root surface

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Transplant into porous soil (soilrite or mix soil and sand)

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Cover the transplanted plants To maintain humidity

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Remove covering gradually

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After proper hardening acclimatize in soil

Transplantation

Application of Micropropagation

- **Produce, maintain, multiply and transport pathogen free plants**
- **Mass clonal multiplication of desirable plant**
- **Multiplication can continue throughout the year irrespective of season.**
- **Virus free plant through meristem culture.**

Limitations

1. Few explants exude dark colored compounds, like phenols, pigments etc which leach into the medium from the cut ends of the explant. It results in the browning of tissues and the medium as well. The browning of medium is associated with poor culture establishment and low regeneration capacity of the explants. This can be **overcome by:**

➢minimizing the wounding of explants during isolation and surface disinfection to reduce this browning response.

➢washing or incubation of explants for 3-5 hrs in sterile distilled water to remove phenolics responsible for browning of medium or explants.

➢Frequent transfer of explants with excision to fresh medium at regular intervals.

➢initial establishment of cultures in liquid medium and later transfer to the semi-solid medium.

➢culture of explants on porous substrate or paper bridges.

➢addition of activated charcoal (AC) or polyvinylpyrrolidone (PVP) for adsorbtion of phenolics.

➢antioxidants like ascorbic acid, citric acid etc. can also be used to prevent browning of tissues in culture.

Limitations

2. Appearance of vitrified tissues (hyperhydricity), a physiological disorder occurring in the *in vitro* cultures due to which the tissues look transparent and fluffy resulting from excessive intake of water. Hyperhydricity can be caused by a high concentration of cytokinin or low concentration of gelling agent or high water retention capacity of explants if the container is tightly closed.

Limitations

3. Loss of regeneration ability in long-term cultures due to epigenetic variations (temporary variations) and culture aging, including transition from juvenile to mature stage. Epigenetic variation are phenotypic temporary variations which disappear as soon as the culture conditions are removed.

Limitations

4. Genotypic variations are also seen in the cultures, therefore, cytological, biochemical and molecular analyses are required to confirm clonal fidelity of *in vitro* regenerants. Besides, morphological and physiological testing is also required to remove undesired genetic variability.