Bharathidasan University
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Programme : M.Sc Biotechnology
Course Title : Plant Biotechnology
Course code :22BTCC12

Unit -1
Micropropagation

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Micropropagation

Micropropagation is a tissue culture technique used for the rapid production of large numbers of genetically identical plants (clones) from a small amount of plant tissue, usually starting from a single explant (a piece of plant tissue) in vitro (in a laboratory setting).

This method is highly beneficial for large-scale plant propagation and for maintaining plant diversity. It is especially useful in agriculture, horticulture, and conservation.

Micropropagation needs young disease free meristematic tissue as starting material. This explant is grown under tissue culture medium enriched with the growth regulator cytokinin or cytokinin with a lesser amount of Auxin.

Micropropagation first initiates the multiple shoots from the explant and these shootsare further multiplied for several subculture phases under cytokinin influence.

The shoots are isolated from the shoot clumps, elongated in shoot elongation medium and rooted with the addition of auxin in tissue culture medium. The rooted plants are acclimatized in soil.

Stages of Micropropagation

- **Key Stages of Micropropagation**

1.Selection of Explant:

1. The first step involves selecting a suitable explant from a healthy parent plant. Explants can be taken from various plant parts such as stems, leaves, roots, meristems, and embryos. The choice of explant depends on the plant species and the desired outcome (e.g., regeneration, root formation).
2. Commonly used explants are shoot tips, nodal segments, or leaves. Meristematic tissues (tissues that are actively dividing) are preferred because they can regenerate a whole plant.

2.Sterilization:

1. Since micropropagation is conducted in a sterile environment, explants are sterilized to eliminate any microorganisms like fungi, bacteria, and viruses. This is typically done by soaking the explants in a disinfectant solution such as sodium hypochlorite (bleach) or ethanol, followed by rinsing with sterile water to remove any residual disinfectant.
2. Proper sterilization is crucial to prevent contamination of the culture medium.

Stages of Micropropagation

3.Initiation of Culture:

After sterilization, the explants are placed on a suitable **culture medium**. The culture medium is typically a gel-like substance (such as agar) containing essential nutrients (macro and microelements), vitamins, amino acids, and a carbohydrate source (commonly sucrose).

Plant growth regulators (PGRs) such as auxins (e.g., indole-3-acetic acid, IAA) and cytokinins (e.g., benzylaminopurine, BAP) are added to the medium to regulate cell division, differentiation, and organ formation. The concentration of these PGRs affects the type of growth response (e.g., shoot or root initiation).

Stages of Micropropagation

4.Shoot Proliferation
(Multiplication Stage):

The explants placed on the culture medium undergo cell division and proliferation. The presence of cytokinin promotes the development of multiple shoots from the explant, a process known as **multiplication**.

This stage is characterized by rapid multiplication of shoots, which can be subcultured (transferred to fresh media) to enhance growth and produce more plantlets.

Stages of Micropropagation

5. Rooting Stage:

After shoot proliferation, the shoots are transferred to a rooting medium containing a higher concentration of auxins, such as indole-3-butyric acid (IBA), to induce root formation.

The explants develop into plantlets with roots, making them capable of being transferred to soil or other growing media.

6. Acclimatization (Hardening Off):

Once the plantlets have formed roots and are growing well in the tissue culture medium, they need to be acclimatized to external conditions. This involves transferring the plantlets from the sterile culture medium to soil or other growing media, such as vermiculite or peat.

Initially, the plantlets are kept in a high humidity environment to prevent desiccation and shock. Gradually, the humidity is reduced, and the plants are allowed to adapt to normal environmental conditions like light, temperature, and air humidity

Micropropagation

Micropropagation can be performed under the following methods

1. Apical meristem culture
2. Axillary shoot proliferation
3. Adventitious shoot production

Among these three methodology Apical meristem culture is the most suitable method. It produces disease free genetically identical plants within the short span of time.

Factors affecting micropropagation

Effects of season on culture establishment

- The extent of contamination as well as bud-break is highly dependent on the season.
- The cultures initiated during spring season (January to April) shows best response not only in terms of the frequency of bud-break but also in the vigor of the shoots with least contamination rate.
- Since, summer (May-August) is the period that concurs with rainy season in certain regions like India, the cultures are prone to infection.
- By winter the shoots become old and it is difficult to break the dormant state of the buds.

Factors affecting micropropagation

ii. Effect of carbon source on shoot proliferation

- In cultured plant tissues, a continuous supply of carbohydrate from the medium is essential which are needed for growth and organized development of the plant and are necessary as a source of energy and carbon skeletons for biosynthetic process.
- For shoot induction from axillary buds, three carbon sources, sucrose, glucose and maltose are utilized in maximum plant tissue cultures at a fixed concentration of 30 g l^{-1} .
- Of these, sucrose is the most commonly used carbohydrate for plant tissue cultures and most culture media have it as the sole carbohydrate source.
- It favours higher growth of shoot, number of nodes per shoot and the rate of shoot multiplication compare to maltose and glucose.
- Sucrose is easily recognized and hydrolysed by cell wall bound invertase into more efficiently utilizable forms of sugars, glucose and fructose which are incorporated into the cells.
- Glucose, derived from sucrose hydrolysis, is more accessible to the cultured tissues than glucose derived by maltose hydrolysis, due to a rapid sucrose hydrolysis but a slow maltose hydrolysis in the media.

Factors affecting micropropagation

iii. Effect of growth regulators on shoot proliferation

- In general, cytokinins favors shoot proliferation and auxins favors root formation.

- The addition of a low concentration of GA_3 to the BAP supplemented medium further promoted multiple shoot formation.

- On the other hand, single shoot with long internodes was developed from axillary buds in cultures when NAA was added to BAP containing medium.

- The frequency of bud-break varied with the concentration of the BAP and at its optimum level of 5 µM, 10-fold shoot multiplication occurred every 5 weeks.

Advantage of Micropropagation

- **Advantages of Micropropagation**

1. **Mass Propagation:** Micropropagation allows the production of large numbers of genetically identical plants (clones) from a single explant in a short period. This is particularly valuable for the mass production of high-quality crops.
2. **Disease-Free Plants:** Plants propagated via micropropagation are usually free from viral, fungal, and bacterial infections. This is especially important for crops susceptible to specific pathogens.
3. **Year-Round Production:** Since the process is carried out in controlled, sterile laboratory conditions, micropropagation can be done throughout the year, independent of seasonal factors.
4. **Preservation of Genetic Resources:** Micropropagation can be used to conserve endangered plant species or to preserve valuable genetic material from elite plants, ensuring that rare or superior plants are not lost.
5. **Shorter Propagation Time:** The time required for propagation is reduced compared to traditional methods, as plants are grown under optimal conditions.
6. **Space Efficiency:** The technique requires little space compared to traditional field-based propagation methods, making it suitable for limited spaces, such as laboratories or greenhouses.

Technical problems of micropropagation

Culture contamination – by bacteria, fungi, viruses is the major problem in micropropagation. Microbial contamination can be controlled by addition of antibiotics or fungicides to the culture medium. Disease free stock plants are maintained to minimize the risk.

Browning of medium – In many species (esp. woody species when explant is taken from mature tree) cut surfaces of explants leads to leaching of phenolics into the medium. Phenolics turn dark brown on oxidation and create condition called as browning of medium which is detrimental to the cultures.

This can be avoided by some do's and don't

Frequent subculturing of explant (about every 37 days)

A brief period of culture in liquid medium (about 3 to 7 days), it is helpful in removing phenolics and other inhibitory substances.

Use of antioxidants (like ascorbic acid or citric acid) may check oxidation of polyphenols.

Use of adsorbents (like charcoal or PVP) to adsorb polyphenols secreted in the medium.

Culturing in dark is another way to prevent polyphenol oxidation as light enhances it.

Application of Micropropagation

1. **Agriculture:** Micropropagation is widely used in the mass production of crops such as bananas, potatoes, tomatoes, and strawberries. It is particularly useful for crops that are difficult to propagate by conventional means (e.g., seeds or cuttings).
2. **Horticulture:** It is used for the commercial propagation of ornamental plants, flowers, and shrubs. Micropropagation allows the rapid production of high-quality plants with desirable traits such as color, shape, and size.
3. **Forestry:** Tree species with desirable characteristics (e.g., disease resistance, growth rate) can be propagated rapidly through micropropagation, helping to replenish forests or establish new plantations.
4. **Conservation:** Endangered and rare plant species can be conserved through tissue culture techniques. Micropropagation helps in the recovery of species that are difficult to propagate sexually or vegetatively in the wild.
5. **Genetic Improvement:** Micropropagation provides a way to introduce new genetic material into a species through techniques such as gene editing or hybridization, leading to the development of superior cultivars.

Limitations of micropropagation

Limitations of Micropropagation

1.Cost: While micropropagation is efficient, the initial setup of laboratory facilities (including sterilization, culture medium preparation, and equipment) can be costly. Moreover, maintaining a sterile environment requires sophisticated infrastructure and expertise.

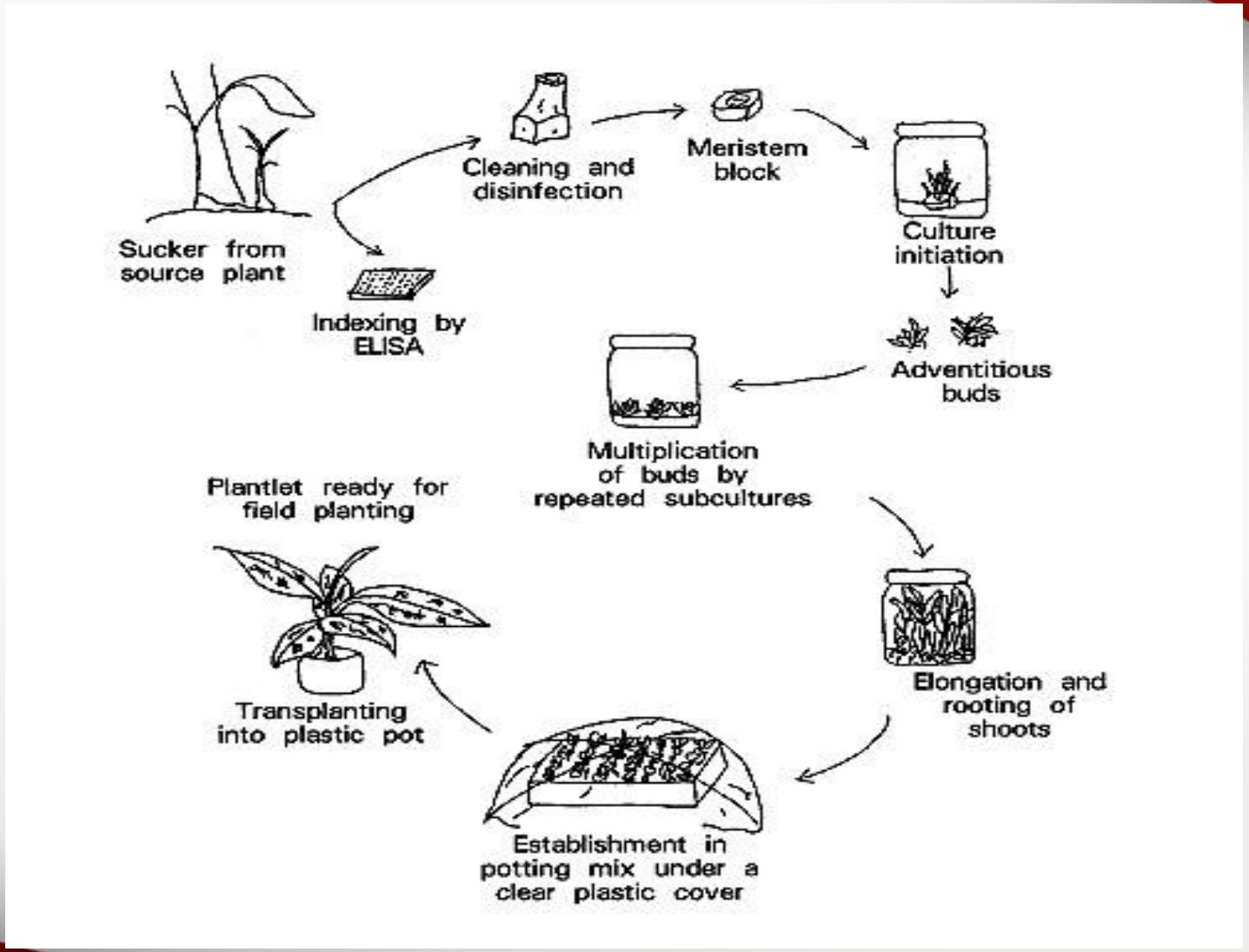
2.Somaclonal Variation: Plants produced through tissue culture may exhibit somaclonal variation (genetic variations that occur due to the tissue culture process itself), leading to differences in plant traits. This variation can be undesirable in commercial crops.

3.Contamination Risk: Despite stringent sterilization techniques, there is still a risk of contamination in tissue cultures, which can spoil entire batches of plants.

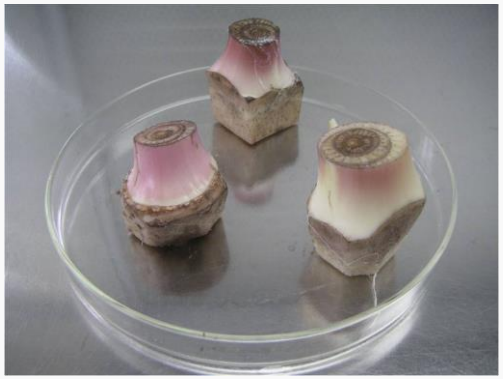
4.Dependency on Plant Growth Regulators: Micropropagation is heavily dependent on the right balance of plant growth regulators. A slight variation in concentration can lead to undesirable results such as poor shoot formation or abnormal growth patterns.

5.Acclimatization Challenges: Successfully acclimatizing tissue-cultured plants to field conditions can be difficult, particularly for certain plant species. Hardening off requires careful monitoring of environmental conditions such as humidity, temperature, and light.

Micropropagation of banana



Micropropagation of banana



References

Smith (2013) Plant Tissue culture : Techniques and Experiments. Academic Press. London, U.K.

Bojwani and Datu (2013): Plant tissue culture : An Introductory Text. Springer, New Delhi, India

Gamborg and Philips (1995)Plant cell Tissue and Organ culture: Fundamental methods Springer Verlag, New York .

Thank you