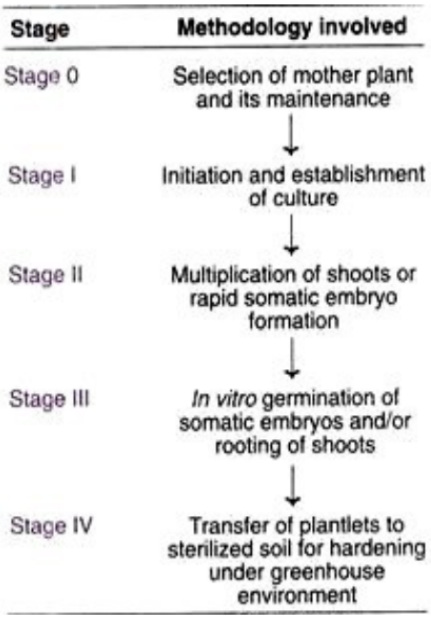
MICROPROPAGATION

Micropropagation

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods

Technique of Micro propagation:

Micro propagation is a complicated process and mainly involves 3 stages (I, II and III). Some authors add two more stages (stage 0 and IV) for more comprehensive representation of micro- propagation. All these stages are represented in the following Figure, and briefly described hereunder.



Major stages involved in micropropagation

Stage 0:

This is the initial step in micro- propagation, and involves the selection and growth of stock plants for about 3 months under controlled conditions.

Stage I:

In this stage, the initiation and establishment of culture in a suitable medium is achieved. Selection of appropriate explants is important. The most commonly used explants are organs, shoot tips and axillary buds. The chosen explant is surface sterilized and washed before use.

Stage II:

It is in this stage, the major activity of micro propagation occurs in a defined culture medium. Stage II mainly involves multiplication of shoots or rapid embryo formation from the explant.

Stage III:

This stage involves the transfer of shoots to a medium for rapid development into shoots. Sometimes, the shoots are directly planted in soil to develop roots. In vitro rooting of shoots is preferred while simultaneously handling a large number of species.

Stage IV:

This stage involves the establishment of plantlets in soil. This is done by transferring the plantlets of stage III from the laboratory to the environment of greenhouse. For some plant species, stage III is skipped, and un-rooted stage II shoots are planted in pots or in suitable compost mixture.

The different stages described above for micro propagation are particularly useful for comparison between two or more plant systems, besides better understanding. It may however, be noted that not all plant species need to be propagated in vitro through all the five stages referred above.

Methods of micropropagation

1. Meristem culture

In meristem culture, meristematic tissues are used as explant for culturing purposes. Meristematic tissues are the type of tissues that can continuously divide and produce new cells. The meristem culture technique was developed by Morel and Martin in 1952 to culture Dahlia. In this case, meristem tissue with a few leaf primordia is placed on a suitable media for the growth and development of plants. The size of the explant is essential to be considered depending on the goal of culturing before initiating the experiment.

Meristem culture technique is best for virus elimination, gene conservation, genetic transformation, and plant breeding purposes.

It has been successfully used to propagate several crops like sugarcane, strawberry, etc.

2. Callus culture

A callus is an undifferentiated mass of tissue. In vitro, the callus formation is induced by placing a piece of tissue in a growth culture media under favourable conditions. Then, the callus is transferred to another fresh media containing a high concentration of auxin or auxin and cytokinin (plant growth regulators) for organ development.

Some ***limitations of callus culture include high biochemical variability and slow growth rate.***

These limitations hinder the utilization of the callus culture techniques at a higher level by the culturists.

3.Suspension culture

The suspension culture method involves the formation of suspension by the multiplication of single cells or aggregates when agitated in an aerated and sterile liquid medium. The two types of suspension culture include batch culture and continuous culture.

In batch culture, cells are grown in a fixed amount of culture medium under suitable environmental conditions. In continuous culture, fresh medium is added and the leftover nutrients are continuously removed from the culture media in which cells are suspended.

The most common problem in this method is the formation of clumps and failure of cells to separate after division.

{Callus and suspension Cultures will be discussed in full at a later lecture}

4. Embryo culture

Embryo culture is the isolation of immature or mature embryos and culture them in a suitable growth media under favorable conditions. The zygotic or seed embryo is used as explant and the presence of nourishing tissue, endosperm, ensures the proper development of the plant. When the endosperms degenerate, as in the case of a cross between two distant species, the development of the embryo is hindered and the plants develop improperly. Therefore, endosperm has an essential role in embryo culture.

To produce healthy plants in cases where endosperms degenerate, the process is followed by culturing the immature hybrid embryo. This process is called embryo rescue. It is done to save embryos that could be aborted.

The embryo rescue technique has massive application in crop improvement.

5. Protoplast culture

Protoplast is a spherical naked living cell without a cell wall. It is obtained by stripping the cell wall of plants by using chemical, mechanical, or enzymatic processes. Protoplast culture is the isolation of plant cells followed by degradation of cell walls and culturing them in a liquid media under suitable physiological conditions. The cultured protoplasts form cell walls followed by calli that are transferred to solid media for the development of the whole plant.

The explants used for protoplast culture are leaves, root tips, and embryos. Out of these three explants, leaves are the common source of protoplast. It’s a bit difficult to strip the cell wall of root tips and embryo and that’s why they aren’t commonly preferred as an explant source for the protoplast culture.

- **Micropropagation** is the tissue culture technique used for rapid vegetative multiplication of ornamental plants and fruit trees. This method of tissue culture

produces several plants. Each of these plants will be genetically identical to the original plant from where they were grown. Well rooted plants are removed from culture vessels and transferred in controlled, but not sterile environmental condition to hardening and subsequent transplantation in the field.

Advantages of micropropagation:

– Rapid multiplication of plants within a short period and on small space.

– Plants are obtained under controlled conditions, independent of seasons.

-Sterile plants or plants which cannot maintain their characters by sexual reproduction are multiplied by this method.

-The rare plant and endangered species are multiplied by this method and such plants are saved.

-Production of virus free plants like potato, sugarcane, banana and apple for horticulture and agriculture.

- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds. This is the main advantage of micropropagation, the production of many plants that are clones of each other.
- Cross distantly related species by protoplast fusion and regeneration of the novel hybrid. (It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.)
- Rapid in vitro selection of stress tolerant plants –salt, drought, heavy metal, low temperature, herbicide resistant
- Regenerate the whole plants from plant cells that have been genetically modified.
- Micropropagation can be used to produce disease-free plants.
- It can have an extraordinarily high fecundity rate, producing thousands of propagules while conventional techniques might only produce a fraction of this number.
- It is useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed cannot be stored.
- Micropropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods - like seeds or cuttings.

- Some plants with very small seeds, including most orchids, are most reliably grown from seed in sterile culture.
- A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.

Disadvantages

Micropropagation is not always the perfect means of multiplying plants. Conditions that limit its use include:

- Labour may make up 50%-69% of operating costs.
- A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
- An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.
- Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant.
- Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
- Some plants are very difficult to disinfect of fungal organisms.

The major limitation in the use of micropropagation for many plants is the cost of production; for many plants the use of seeds, which are normally disease free and produced in good numbers, readily produce plants in good numbers at a lower cost. For this reason, many plant breeders do not utilize micropropagation because the cost is prohibitive. Other breeders use it to produce stock plants that are then used for seed multiplication.

Mechanisation of the process could reduce labour costs, but has proven difficult to achieve, despite active attempts to develop technological solutions.

Selection of mother plant

In vitro culture of plants in a controlled, sterile environment

Micropropagation begins with the selection of plant material to be propagated. The plant tissues are removed from an intact plant in a sterile condition. Clean stock materials that are free of viruses and fungi are important in the production of the healthiest plants. Once the plant material is chosen for culture, the collection of explant(s) begins and is dependent on the type of tissue to be used; including stem tips, anthers, petals, pollen and other plant tissues. The explant material is then surface sterilized, usually in multiple courses of bleach and alcohol washes, and finally rinsed in sterilized water. This small portion of plant tissue, sometimes only a single cell, is placed on a growth medium, typically containing sucrose as an energy source and one or more plant growth regulators (plant hormones). Usually, the medium is thickened with agar to create a gel which supports the explant during growth. Some plants are easily grown on simple media, but others require more complicated media for successful growth; the plant tissue grows and differentiates into new tissues depending on the medium. For example, media containing cytokinin are used to create branched shoots from plant buds.

Multiplication

Multiplication is the taking of tissue samples produced during the first stage and increasing their number. Following the successful introduction and growth of plant tissue, the establishment stage is followed by multiplication. Through repeated cycles of this process, a single explant sample may be increased from one to hundreds and thousands of plants. Depending on the type of tissue grown, multiplication can involve different methods and media. If the plant material grown is callus tissue, it can be placed in a blender and cut into smaller pieces and recultured on the same type of culture medium to grow more callus tissue. If the tissue is grown as small plants called plantlets, hormones are often added that cause the plantlets to produce many small offshoots. After the formation of multiple shoots, these shoots are transferred to rooting medium with a high auxin\cytokinin ratio. After the development of roots, plantlets can be used for hardening.

Pretransplant

Banana plantlets transferred to soil (with vermicompost) from plant media. This process is done for acclimatization of plantlets to the soil as they were previously grown in plant media. After growing for some days, the plantlets are transferred to the field.

This stage involves treating the plantlets/shoots produced to encourage root growth and "hardening." It is performed *in vitro*, or in a sterile "test tube" environment.

"Hardening" refers to the preparation of the plants for a natural growth environment. Until this stage, the plantlets have been grown in "ideal" conditions, designed to encourage rapid growth. Due to the controlled nature of their maturation, the plantlets often do not have fully functional dermal coverings. This causes them to be highly susceptible to disease and inefficient in their use of water and energy. In vitro conditions are high in humidity, and plants grown under these conditions often do not form a working cuticle and stomata that keep the plant from drying out. When taken out of culture, the plantlets need time to adjust to

more natural environmental conditions. Hardening typically involves slowly weaning the plantlets from a high-humidity, low light, warm environment to what would be considered a normal growth environment for the species in question.

Transfer from culture

Plant tissue cultures being grown at a USDA seed bank, the National Centre for Genetic Resources Preservation.

In the final stage of plant micropropagation, the plantlets are removed from the plant media and transferred to soil or (more commonly) potting compost for continued growth by conventional methods.

This stage is often combined with the "pretransplant" stage.

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