Aim: To study the tissue culture technique- Micropropagation

Introduction:

Micropropagation is the tissue culture technique used for rapid vegetative multiplication of ornamental plants and fruit trees. **Frederick Campion Steward** discovered and pioneered micropropagation and plant tissue culture in the late 1950s and early 1960s. Micropropagation produces several plants. Each of these plants will be genetically identical to the original plant from where they were grown. This method of tissue culture is an integrated process in which cells, tissues or organs of the selected plants are isolated, surface sterilized and incubated in growth promoting, sterile medium and environment to produce a large number of plants.

Stages in Micropropagation:

There are four important stages in the process of Micropropagation along with a stage of selecting the mother plant to start the process with

Stage 0: Selection of mother plant for explants isolation:

The mother plant from which explants has to be excised should be a certified and true to type representative of the desired species and or cultivar; Healthy and free from insect pest and disease and Should be quite vigorous.

Stage I - Establishment

During the establishment stage, the explant must be disinfested and stabilized. The explant is usually sterilized with a combination of detergent and bleach. In difficult situations, alcohol or a fungicide may be used. The objective of this stage is to get clean cultures that can begin the process of shoot multiplication. The explant is cultured in a suitable culture medium, preferably agar based media for tissue activation and multiplication.

Stage II - Shoot Multiplication

The objective of the shoot multiplication stage is to increase the number of shoots produced by the original explant. In this stage, repeated sub-culturing to new medium is done to encourage more proliferation and the number of shoots produced in culture increases. A high cytokinin to auxin ratio is used during the multiplication stage to induce axillary or adventitious shoot formation. This ratio is decided upon by preliminary research. Too high a concentration of cytokinin will result in a high number of adventitious shoots that do not elongate. Common cytokinins used in culture are benzyladenine and kinetin.

Stage III - Root formation

Shoots multiplied in culture must be rooted in order to create a new plantlet. In this stage the selected plants are forced for root formation, which can be achieved by media modification and modifying the concentration of growth regulators usually by application of auxin. The concentration of cytokinins and sugars are reduced and concentration of auxins and light intensity is increased to start with photosynthesis and other physiological activities. Microcuttings are inserted directly into the rooting substrate often using forceps to handle the small cuttings.

Stage IV - Acclimatization

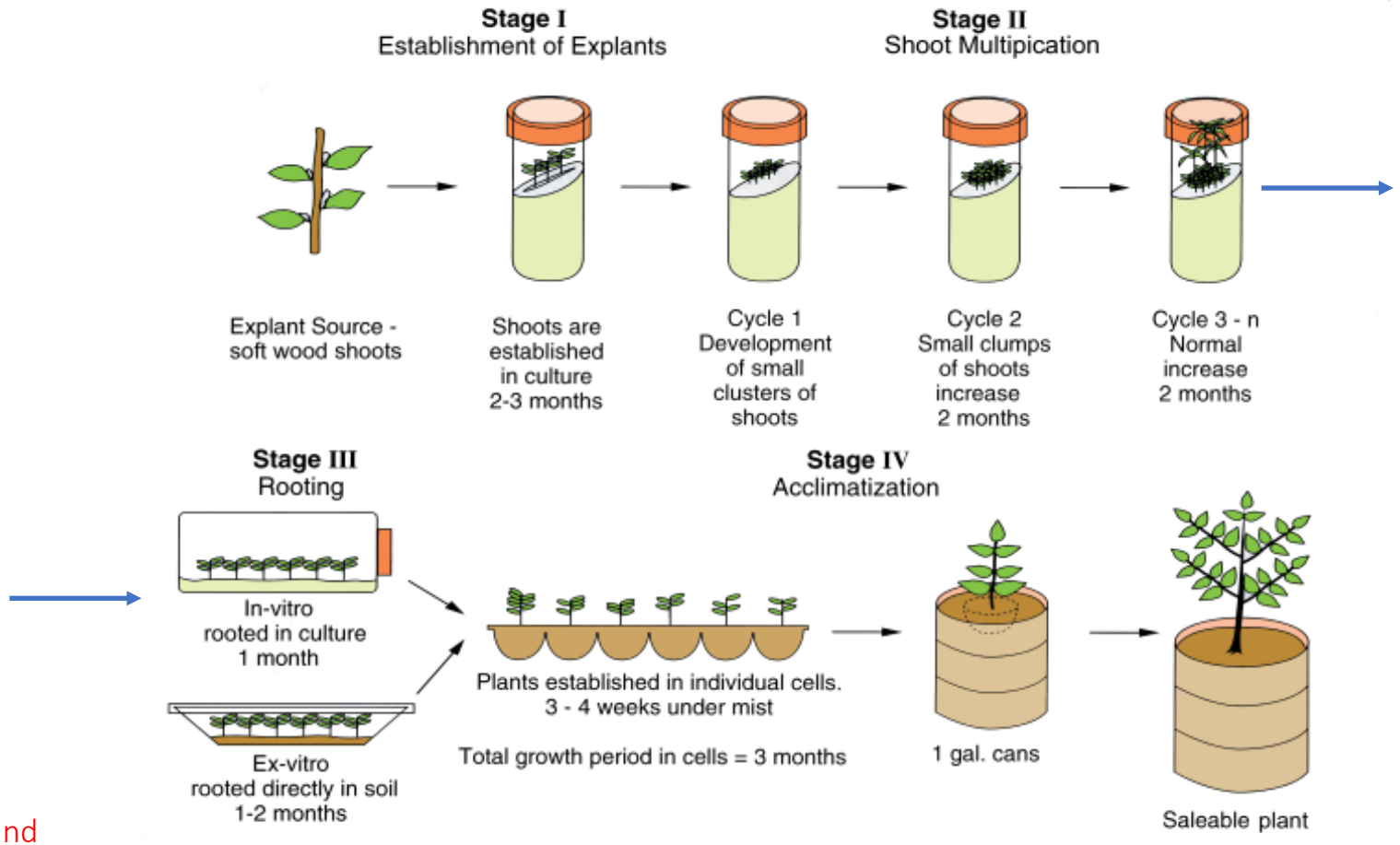
Finally, after roots have become well established on the microcutting, plantlets must be acclimatized to a normal growing environment. This involves gradually moving to open-air conditions where the humidity is reduced and the light levels increased. This is a vulnerable stage for plantlet survival. The roots developed in vitro are hairless and hence delicate, requiring care during transfer from culture medium. To have better survival rate, the plantlets may be transferred to container kept in mist chambers where relative humidity is maintained at higher order. Once new growth is seen, the plants may be slowly transferred to outside by exposing to increased light intensity in stages.
Once plantlets are well rooted, they must be acclimatized to the green house environment. These conditions help the plantlets in getting acclimatized to the natural conditions, which would help them in getting established in the field easily.

Advantage of Micropropagation:

- The main advantage of micropropagation is the production of many plants that are clones of each other.
- 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 the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
- 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.

Disadvantages of Micropropagation:

- The technique is very expensive
- 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.
- 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.



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Stages during the process of Micropropagation

Stage	Methodology involved
Stage 0	Selection of mother plant and its maintenance
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Stage I	Initiation and establishment of culture
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Stage II	Multiplication of shoots or rapid somatic embryo formation
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Stage III	*In vitro* germination of somatic embryos and/or rooting of shoots
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Stage IV	Transfer of plantlets to sterilized soil for hardening under greenhouse environment

Major stages involved in micropropagation.

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