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Developmental Stages of Micropropagation

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SUMMARY

Micropropagation is an advanced in vitro technique for propagating plant genotypes true-to-type through methods such as enhanced axillary shoot proliferation, adventitious shoot formation, micrografting and nonzygotic embryogenesis. The process involves several critical stages: selecting and preparing donor plants under controlled conditions, establishing aseptic cultures by managing timing and explant conditions, proliferating axillary shoots with optimal cytokinin levels, preparing shoots for soil transfer, and finally acclimating plantlets to greenhouse conditions. Successful micropropagation requires meticulous management of these stages to optimize plant growth, minimize costs, and ensure effective transition from in vitro to ex-vitro environments.

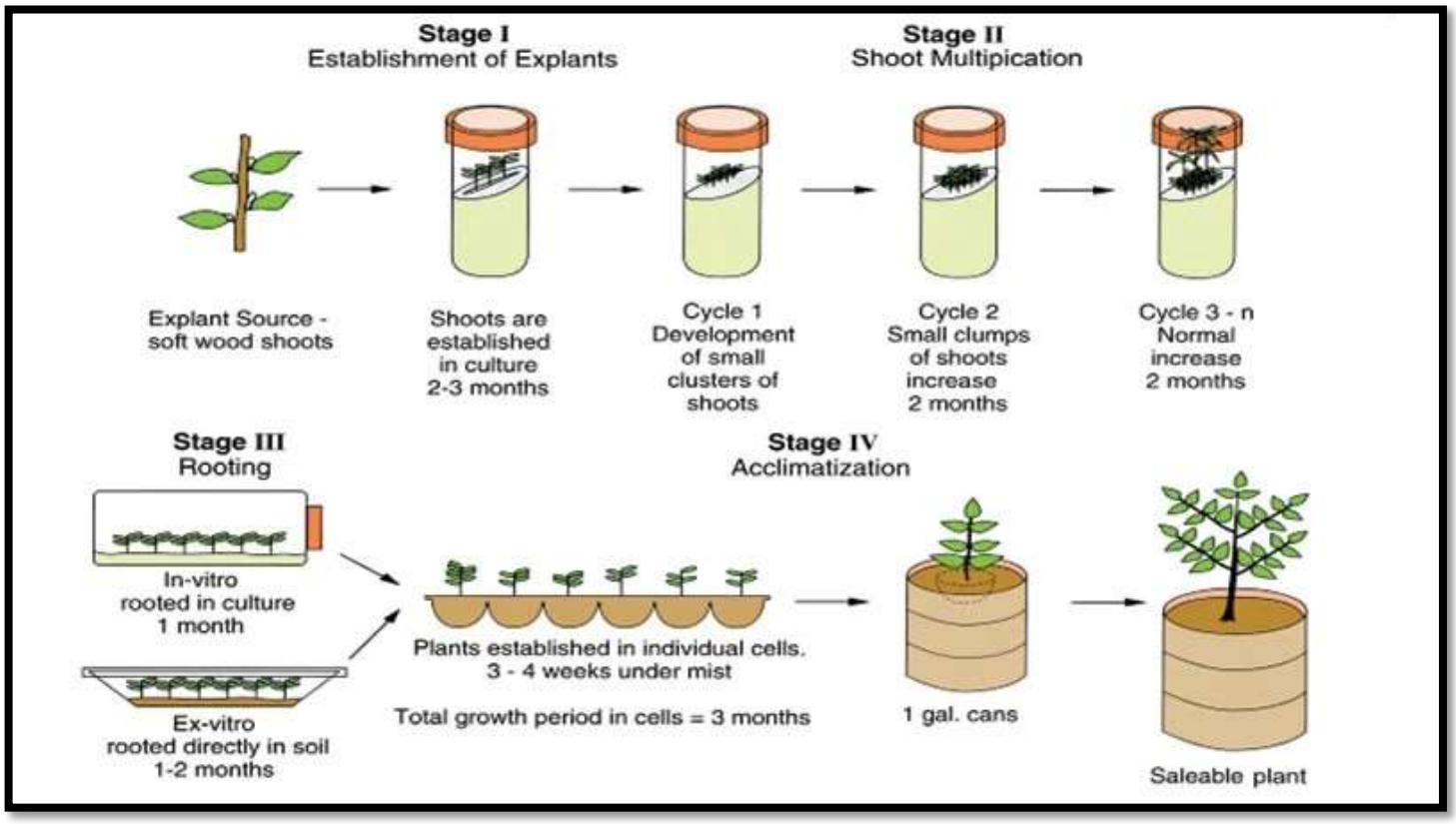
INTRODUCTION

Micropropagation is defined as the true-to-type propagation of selected genotypes using in vitro techniques. Four basic methods are used to propagate plants in vitro depending on the species and cultural condition, micropropagation can be achieved by:

1. **Enhanced axillary shoot proliferation (shoot culture) Suitable explants**: Shoot tip, meristem tip, nodal culture, stool shoot, proliferation of pseudo-corms and mini-tubers
2. **Adventitious shoot formation Suitable explants:** inter-node section, leaf pieces, fragmented shoot apices, immature inflorescence and bulb scale
3. **Micrografting Suitable explants**: Shoot/meristem tip
4. **Nonzygotic embryogenesis:** Suitable explants: cell, nucellus tissue, callus and shoot tip

Developmental stages in micropropagation

Micropropagation is the art and science of plant multiplication. *In vitro* success of micropropagation largely depends on the stages of stock plant preparation and subsequent culture preparation, establishment, morphogenesis and acclimatization of developed plants. Each stage is subjected to specific cultural, nutritional, hormonal and environmental conditions. Based on the various research information, it is now established that there are five major stages (0 to 4) that are critical for the execution of successful plant regeneration.



Stage 0: Donor plant selection and preparation

Explant quality and subsequent responsiveness in vitro are significantly influenced by the phytosanitary and physiological conditions of the donor plant (Debergh and Maene, 1981). Before culture establishment, careful attention is given to the selection and maintenance of the stock plants used as the source of explants. Stock plants are maintained in clean, controlled conditions that allow active growth but reduce the probability of disease. Maintenance of specific pathogen-tested stock plants under conditions of relatively lower humidity and use of drip irrigation and antibiotic sprays have proved effective in reducing the contamination potential of candidate explants. Such practices also allow excision of relatively larger and more responsive explants often without increased risks of contamination.

Numerous practices are employed to increase explant responsiveness by modifying the physiological status of the stock plant. These practices include the following: (1) trimming to stimulate lateral shoot growth; (2) pretreatment sprays containing cytokinins or gibberellic acid; and (3) use of forcing solution containing 2% sucrose and 200 mg/L 8- hydroxyquinoline citrate for induction of bud break and delivery of growth regulators to target explant tissues (Singh *et al.*, 2021). Currently, information on the effect of other factors such as stock plant nutrition, light and temperature treatments on the subsequent in vitro performance of meristem explants is lacking.

Stage 1: Establishment of aseptic cultures

Initiation and aseptic establishment of pathogen eradicated and responsive terminal or lateral shoot meristems explants are the goal of this stage. The primary explants obtained from the stock plants may consist of surface disinfested shoot apical meristems or meristem tips for pathogen elimination or shoot tips from terminal or lateral buds

The factors may affect successful stage I establishment of meristem explants are: explantation time, position of the explant on the stem, explant size and polyphenol oxidation.

Time of explantation can significantly affect explant response in- vitro. In deciduous woody perennials, shoot tip explants collected at various times during the spring growth flush may vary in their ability for shoot proliferation. Shoot tips collected during or at the end of the period of most rapid shoot elongation exhibited weak proliferation potential. Explants collected before or after this period is capable of strong shoot proliferation in vitro (Brand, 1993). Conversely, the best results are obtained with herbaceous perennials that form storage organs, such as tubers or corms, when explants are excised at the end of dormancy and after sprouting.

Explants also exhibit different capacities for establishment in vitro depending on their location on the donor plant. For example, survival and growth of terminal bud explants are typically greater than lateral bud

explants. Often similar lateral meristem explants from the top and bottom of a single shoot may respond differently in-vitro in woody plants exhibiting phasic development, juvenile explants typically are often more responsive than those obtained from the often non-responsive mature tissues of the same plant. Sources of juvenile explants include the following:

(1) Root suckers
(2) Basal parts of mature trees
(3) Stump sprouts
(4) Lateral shoots produced on heavily pruned plants.

The excision of primary explants often promotes the release of polyphenols and stimulates polyphenol oxidase activity within the damaged tissues. Oxidized polyphenol products often blacken the explant tissue and medium. Accumulation of these products can eventually kill the explants. Procedures used to decrease tissue browning include

(1) Use of liquid medium with frequent transfer
(2) Adding antioxidants such as ascorbic acid or polyvinylpyrrolidone (PVP)
(3) Culture in reduced light or darkness.
(4) Season of explantation.

There clearly is no one universal culture medium for establishment of all species. However, modifications to the Murashige and Skoog (Murashige and Skoog, 1962) basal medium formulation are most frequently used. Cytokinins or auxins are most frequently added to stage I media to enhance explant survival and shoot development (Hu and Wang, 1983). The types and levels of growth regulators used in stage I media are dependent on the species, genotype and explant size.

Knowledge of the specific sites of hormone biosynthesis in intact plants provides insight into the relationship between explant size and dependence on exogenous growth regulators in the medium. Endogenous cytokinins and auxins are synthesized primarily in root tips and leaf primordia, respectively. Consequently, smaller explants, especially cultured apical meristem domes, exhibit greater dependence on medium supplementation with exogenous cytokinin and auxin for maximum shoot survival and development (Shabde and Murashige, 1977). Larger shoot tip explants usually do not require the addition of auxin in Stage I medium for establishment. Rapid adventitious rooting of shoot tip explants often provides a primary endogenous cytokinin source.

Stage II: Proliferation of axillary shoots

Stage II propagation is characterized by repeated enhanced formation of axillary shoots from shoot tips or lateral buds cultured on a medium supplemented with a relatively higher cytokinin level to disrupt apical dominance of the shoot tip. A subculture interval of 4 weeks with a three to eightfold increase in shoot number is common for many crops propagated by shoot culture. Given these multiplication rates, conservatively, more than 4.3 x 107 shoots could be produced yearly from a single starting explant.

Stage II cultures are routinely subdivided into smaller cluster, individual shoot tips or nodal segments that serve as propagules for further proliferation. Additionally, axillary shoot clusters may be harvested as individual unrooted Stage II micro cuttings or multiple shoot clusters for ex vitro rooting and acclimatization. Clearly, Stage II represents one of the costliest stages in the production process.

Both source and orientation of explants can affect Stage II axillary shoot proliferation. Subcultures inoculated with exhibit higher multiplication rates than lateral bud explants. Inverting shoot explants-oriented explants per culture period in some species.

The number of subcultures possible before initiation of new Stage II cultures from the mother block is required depends on the species or cultivar and its inherent ability to maintain acceptable multiplication rates while exhibiting minimal genetic variation and off –types (Kurtz et al., 1991). Some species can be maintained with monthly subculture from 8 to 48 months in Stage II. In contrast, in some ferns (*Nephrolepsis*), as few as three subcultures may only be possible before the frequency of off-types increases to unacceptable levels.

Stage III: Pre-transplant (rooting)

This step is characterized by preparation of Stage II shoots or shoot clusters for successful transfer to soil. The process may involve:

(1) Elongation of shoots prior to rooting
(2) Rooting of individual shoots or shoot clumps

(3) Fulfilling dormancy requirements of storage organs by cold treatment
(4) Prehardening cultures to increase survival.

Where possible commercial labourites have developed procedures to transfer stage II micro cuttings to soil, thus bypassing stage III rooting. There are several reasons for eliminating Stage III rooting. Estimated costs for stage III range from 35 to 75% of the total production costs. This reflects the significant input of labour and supplies required to complete stage III rooting. Considerable cost saving can be realized if stage III is eliminated. Furthermore, it is often observed that in vitro formed root systems are largely nonfunctional and die following transplanting. This results in a delay in transplant growth prior to production of new adventitious roots.

Stage IV: Hardening of plantlets

The ultimate success of micropropagation depends on the ability to transfer and reestablish vigorously growing plants from in vitro to greenhouse conditions. This involves acclimatizing or hardening –off plantlets to conditions of lower humidity and higher light intensity. Even when acclimatization methods are carefully followed, poor survival rates are achieved. Micropropagated plants are difficult to transfer in vivo conditions due to reasons such as heterotrophic mode of nutrition and poor control of water loss.

Plants cultured in vitro in the presence of sucrose and under conditions of limited light and gas exchange exhibit no or extremely reduced capacities for photosynthesis. Reduced photosynthetic activity is associated with poor stomatal functioning. During acclimatization, there is a need for plants to rapidly transition from the heterotrophic state for survival (Preece and Sutter,1991). Photosynthetic competency Interestingly, before senescence, these older leaves function as “lifeboats” by supplying stored carbohydrates to the developing and photosynthetically competent new leaves. This is not the rule with all micro-propagated plants since the leaves of some species become photosynthetic and persist after acclimatization.

For successful acclimatization of the plant lets the following infrastructural facilities are required.

1. Pots of different sizes
2. Potting mixtures such as vermiculite, perlite, cocopeat, and vermicompost
3. Hardening chamber fitted with temperature, photoperiod, and humidity control devices
4. Poly house/greenhouse facility with controlled temperature and humidity factors
5. Shade house/ Net house with fogging/misting devices
6. Shade house for further growth and development of plantlets in containers filled with a mixture of sand, soil and compost.

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