STUDY MATERIAL

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BOTANY

(HONOURS)

Stages of Plant Micropropagation

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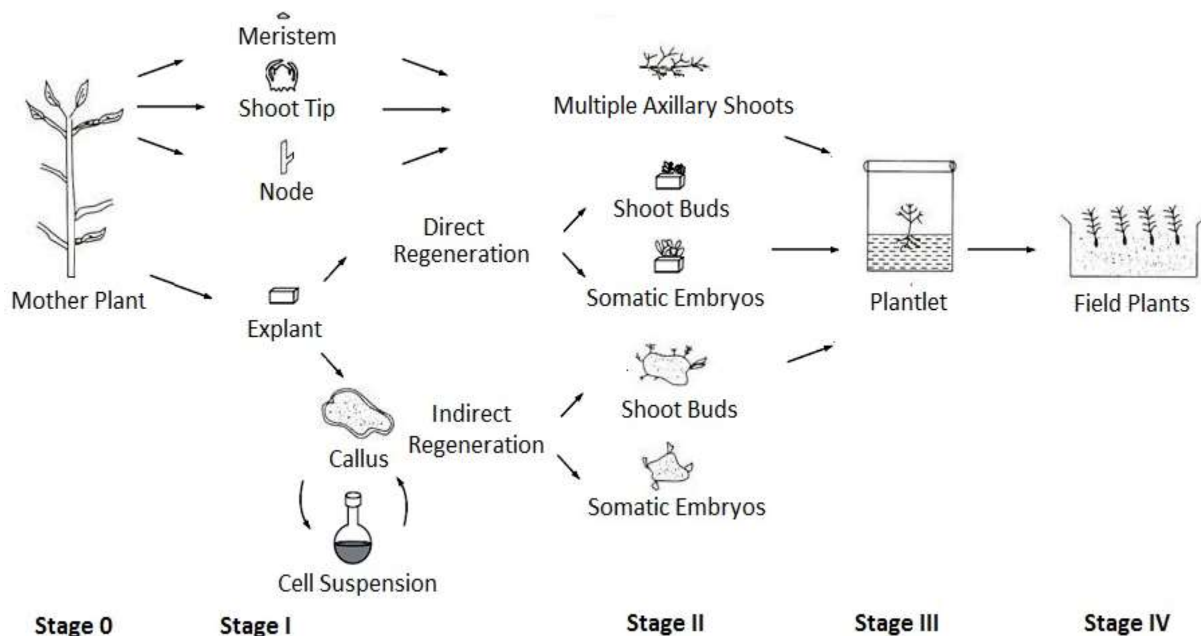
Plant Micropropagation

Micropropagation, also known as ***in vitro*** **propagation**, is the ***in vitro*** technique of using **small propagules** in the form of isolated plant cells, tissues or organs (*i.e.* **explants**) for the **asexual propagation** of plants in plant tissue culture.[1] Micropropoagation, in a strict sense, involves multiplying **pre-existing meristems** in the form of **apical or axillary meristems**. However, in a wider sense, it also includes **plant regeneration** from **totipotent cells**[2] directly on the explant as **adventive** structures or indirectly from their callus as ***de novo*** structures, both in the form of **shoots** or **embryoids**. Micropropagation is one of the most important practical applications of plant tissue culture. Formation of **true-to-type plants** is called **clonal propagation**. This is used for the rapid, large-scale propagation of economically-important taxa, as well as for the ***ex situ*** **conservation** of endangered ones. However, the presence of a **callus**[3] phase can cause genetic variability (**somaclonal variation**[4]) but this can also be exploited for the improvement of economically-important taxa.

Stages of Micropropagation

Toshio Murashige defined three **stages (I-III)** of *in vitro* plant propagation (1974). Debergh and Maene (1981) added a preparatory **stage (0)** and a fourth **stage (IV)** of transferring the plants to an external environment. These **four stages** are as illustrated below (Fig. 1).

Figure 1: Schematic diagram of an overview of plant micropropagation.



(Modified from George *et al*., 2008).

[1] Plant tissue culture is a collection of *in vitro* techniques used to maintain, grow or manipulate plant cells, tissues or organs in artificial nutrient medium, under axenic and controlled conditions.
[2] Cellular totipotency is the genetic potential of a plant cell to develop into a complete plant.
[3] Callus is an unorganized proliferating mass of cells produced from isolated plant cells, tissues or organs during *in vitro* culture.
[4] Somaclonal variation is the genetic variability produced during plant tissue culture.

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Stage 0: Mother Plant Selection and Preparation

Prior to micropropagation, the **stock plants** are collected and grown for at least 3 months under **glasshouse conditions** free of both **abiotic and biotic stresses**. The selected plants must be **typical** of the **taxa**, **young** and **healthy**. This **preconditioning stage** involves maintaining the stock plant under normal **physiological conditions** with reduced **surface and systematic microbial contaminants**. Micropropagation can also be significantly improved by appropriate physical or chemical pre-treatment of these stock plants.

Stage I: Establishment of Aseptic Culture

The first step in micropropagation is to obtain an aseptic *in vitro* culture of the selected plant material. A suitable **explant** is freed from **microbial contaminants** and transferred to an appropriate *in vitro* cultural environment for some kind of growth (such as the growth of a shoot tip, axillary bud or root tip, or formation of callus) in the presence of a suitable **nutrient medium** and under appropriate **controlled conditions**. The explant selection and preparation is critical for achieving **asepsis** (Stage I) and must contain adequate **meristems** or **totipotent cells** for the multiplication of propagules (Stage II). The explant is decontaminated by an optimal suitable **surface sterilization** protocol that causes maximum damage to the microbial contaminants but with minimum damage to the plant tissues. This is done in **batches**. After incubation, the cultures with contaminated explants or medium are discarded. Stage I involves at least three **passages** of **subculture** and it is satisfactorily completed only if an adequate number of explants survived without contamination, and keeps growing.

Stage II: Multiplication of Propagules

The objective of Stage II is to produce new plant outgrowths *i.e.* **plant propagules**, which can be separated from the culture and converted into complete plants. These propagules are in the form of **multiple axillary shoots**, **adventive** or ***de novo*** **shoots**[5] and **somatic embryos**[6] (and rarely **miniature storage organs** such as corms, bulbs and tubers). This multiplication step of *in vitro* propagation uses a wide array of methods as summarized above (Fig. 1). There are two broad approaches for the *in vitro* multiplication of propagules. One approach simply involves multiplying the **pre-existing meristems** at the tips (**apical meristem**) and nodes (**axillary meristem**) of the shoots. Thus no plant regeneration (or callus formation) in involved. The explants are in the form of apical or axillary meristems (**meristem culture**), shoot tips (**shoot culture**) or single or multiple nodes (**node culture**). These are usually treated with **cytokinins** such as **BA** (6-benzyladenine), **2iP** (2-isopentenyladenine) or **kinetin** in the culture medium to produce multiple axillary shoots. The other approach involves the induction of **adventive** or ***de novo*** structures such as **shoots** or **embryoids** through **plant regeneration** of **totipotent cells** present directly on the explant (**direct regeneration**) or indirectly from their **callus tissue** (or **cell suspension**) through **dedifferentiation** (**indirect or callus-mediated regeneration**). The explants are usually treated with either cytokinins (such as those above) alone or in combination with **auxins** such as **IAA** (indole-3-acetic acid), **IBA** (indole-3-butyric acid) or **NAA** (naphthalene acetic acid), or the auxin **2,4-D** (2,4-dichlorophenoxyacetic acid) in the culture medium to produce adventive or *de novo* shoots and somatic embryos.

[5] Shoot organogenesis produces shoot primordia that develop into shoot buds and shoots.

[6] Somatic embryogenesis produces pro-embryos that develop through the globular, heart-shaped and torpedo stages into cotyledonary (mature) somatic embryos.

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Stage III: Growth and Rooting of Propagules

The **multiplied propagules** derived from Stage II are small, immature (and often not complete as in the case of shoots) and therefore incapable of self-supporting growth when transferred to soil or compost in the **external environment**. During Stage III, the final *in vitro* step in micropropagation, measures need to be taken to separate the multiplied propagules and convert them into **compete *in vitro* plantlets** that are capable of survival without an artificial support under **field conditions** during Stage IV. Sometimes, the plantlets need to be specially treated at this stage so that they do not become dormant or stunted when taken out of the cultural environment. The **multiple shoots** are **excised**, **separated** and transferred to a nutrient medium supplemented with auxins such as **IAA** (indole-3-acetic acid), **IBA** (indole-3-butyric acid) or **NAA** (naphthalene acetic acid) for their **elongation** and **rooting** to form complete *in vitro* plantlets. Sometimes, the shoots may need to be elongated (**Stage IIIA**) before rooting (**Stage IIIB**). In contrast, the somatic embryos are bipolar and are transferred to nutrient medium supplemented with **ABA** (abscisic acid) or **GA_3** (gibberellic acid) for their **maturation** and direct **germination** into complete *in vitro* plantlets. These plant growth regulators prevent the unwanted **precocious germination** of the developing somatic embryos. Sometimes, the Stage III can be omitted and the shoots from Stage II can be rooted during hardened in the exterior environment in the final stage (IV).

Stage IV: Hardening and Transfer to the Field

In the last stage (IV) of micropropagation, the **complete *in vitro* plantlets** from the previous stage (III) are **transferred** out of the controlled **cultural environment** into the **external environment** of the green house or the field. This step is of extreme practical importance because if it is not carried out carefully then this can result in a significant **loss of the propagated material**. The main reasons for this are that the *in vitro* plantlets are produced in an **axenic**, **high humidity** and **low light** conditions and are acutely dependent on an **external carbon source**. This results in reduced leaf **cuticular wax**, often with an altered chemical composition in comparison with the normal field plants. In some plants, the **atypical stomata** of leaves produced *in vitro* are incapable of complete closure under the external conditions of low relative humidity. Tissue cultured plants are therefore **susceptible** to **pathogen attack** and **loss of water** when moved to the external conditions. When supplied with sucrose and kept in low light conditions, micropropagated plantlets are not fully dependent on their own photosynthesis (*i.e.* they are **mixotrophic**). A stimulus which is not provided in the *in vitro* environment seems to be needed for them to change and become fully **phototrophic**. The change only occurs after the plants have spent a period of several days *ex vitro*. Thus, the plants need to be gradually **acclimatized** to the field conditions and fully **hardened** for the **field transfer**. In practice, the plantlets are removed from their Stage III containers and the solid agar medium is carefully washed from the roots. The application of an **anti-transpirant** film on the leaves is recommended at this stage. The plantlets are also treated with a **systemic fungicide**. The plantlets are finally transplanted into a suitable sterilized **potting mixture** such as **peat-sand** or **soil-sand** mixed with **compost** or **vermiculite** and kept for several days in high humidity and reduced light intensity inside a **green house**. Intermittent water misting may be applied, or the plants are placed inside clear **plastic bags**. Sometimes, the Stage III is omitted to reduce the time span of the micropropagation cycle, and the shoots from Stage II are rooted directly in the soil or compost under high humidity conditions, and at the same time gradually hardened in the exterior environment.

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Further Reading

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