Chapter-3

Plant Tissue Culture: Micropropagation, Organogenesis and Embryogenesis.

Sonika Kujur[1], Anjali Swain[2], Dr. R. Pushpa[3] and Dr. Samarendra Narayan Mallick[4]

[1]Regional Institute of Education, Bhubaneswar, Odisha, India.
[2]Department of Biotechnology, NIIS Institute of Information Science and Management, Bhubaneswar, Odisha, India.
[3]Plant Breeding and Genetics, Tamil Nadu Rice Research Institute, Aduthurai, Thanjavur District, Tamil Nadu
[4]Department of Botany, Ravenshaw University, Cuttack, Odisha, India.*
samarendra.mallick1@yahoo.com

ABSTRACT

Rapid clonal plant propagation under sterile circumstances is possible with the help of the potent plant tissue culture technology known as micropropagation. In micropropagation, organogenesis and somatic embryogenesis are two important developmental mechanisms. Organogenesis is the process by which explants or callus tissues develop into organs like shoots and roots. This process is usually brought about by a carefully regulated ratio of auxins and cytokinins, which are plant development regulators. On the other hand, somatic embryogenesis, which is similar to zygotic embryogenesis but does not involve fertilization, results in the creation of embryos from somatic or non-reproductive cells. For the large-scale propagation of elite or genetically modified plants, genetic conservation, and plant regeneration, both methods provide clear benefits. Enhancing the effectiveness and suitability of

micropropagation in horticulture, forestry, and agriculture requires an understanding of the physiological and molecular mechanisms that underlie these routes.

KEYWORDS: Micropropagation, organogenesis, embryogenesis.

INTRODUCTION

A contemporary method of plant tissue culture called micropropagation is utilized to quickly and extensively multiply plants in sterile lab settings. This technique makes use of plant cells' totipotency, which is the extraordinary capacity of a single cell to divide into an entire organism. It is particularly helpful for growing plants that are hard to cultivate from seeds or cuttings using traditional methods. Because of its effectiveness, speed, and capacity to create genetically identical and disease-free plants, micropropagation is used extensively in horticulture, forestry, agriculture, and plant biotechnology.

An explant, which is a tiny piece of the parent plant, like a shoot tip, leaf, or node, is carefully chosen at the start of the procedure. After being cleaned of all microorganisms with chemical disinfectants, this explant is put on a culture medium that is high in nutrients. Essential minerals, vitamins, carbohydrates, and plant growth regulators are all included in the medium, which is frequently based on the Murashige and Skoog (MS) formulation. These regulators, which are mostly auxins and cytokinins, are essential for controlling the explant's growth and determining whether it will produce callus, roots, or shoots. To guarantee ideal growing circumstances, the tissue culture is kept in a controlled environment with adjustable humidity, light, and temperature.

Organogenesis and somatic embryogenesis are the two main developmental mechanisms in micropropagation. The process of organogenesis involves the direct or indirect formation of new plant organs from the explant, such as roots and shoots.

This procedure can take place indirectly through an intermediate callus stage, or directly, where organs emerge from the explant without callus development. On the other hand, somatic embryogenesis is the process by which embryos are created from somatic (non-reproductive) cells. Although they are created asexually, these embryos resemble the normal embryonic development that takes place in seeds. This technique is particularly helpful for genetic engineering, the manufacture of synthetic seeds, and cryopreservation for long-term storage.

MICROPROPAGATION

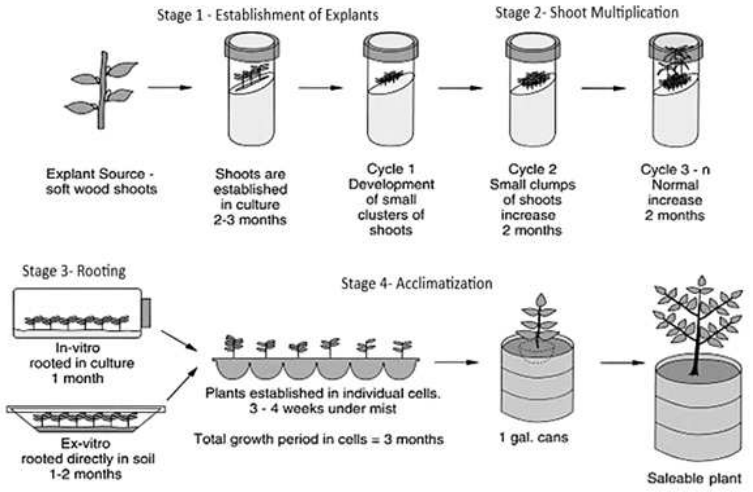

Figure 1. Stages of micropropagation.

The four primary phases of micropropagation are usually initiation, multiplication, rooting, and acclimation. The sterile explant is placed on a culture medium and starts to grow during the initiation stage. Repeated subculturing is used to induce and multiply shoots or embryoids during the multiplication stage. To encourage the production of roots, the developing shoots are moved to a medium rich in auxin during the rooting stage. Finally, in a process known as hardening, the plantlets

are progressively acclimated to outdoor circumstances, initially in a greenhouse and then in open fields or pots. Making the switch from regulated to wild settings is essential to the propagated plants' survival and well-being.

There are many benefits to micropropagation. It is perfect for commercial growing because it can produce a lot of plants in a short amount of time. Additionally, it guarantees genetic homogeneity, which is necessary to preserve desired crop qualities. Additionally, the procedure can be used to eradicate infections and create planting material free of viruses, which is crucial for crops like potatoes, sugarcane, and bananas. Because micropropagation allows for quick multiplication and preservation, it also helps conserve rare, endangered, or slow-growing organisms. Notwithstanding its many advantages, the method has certain drawbacks, such as high setup costs, the requirement for trained labor, and the possibility of contamination or somaclonal variation in the event that cultures are not adequately maintained. To sum up, micropropagation is a major development in plant biotechnology. Its rapid and efficient production of homogeneous, disease-free, high-quality plants has revolutionized the propagation of plants for commercial, conservation, and scientific uses. Micropropagation is anticipated to become even more significant in sustainable agriculture and environmental preservation as technology advances.

PROCESS

- Micropropagation relies on the totipotency of plant cells – the ability of a single cell to develop into a complete plant.
- The technique utilizes explants (small sections of plant tissue), such as shoot tips, nodes, leaves, or even single cells.
- These explants are cultured in a nutrient-rich medium containing plant growth regulators (PGRs) like auxins and cytokinins.

- Under controlled environmental conditions (light, temperature, humidity), the explant multiplies and differentiates into whole plants.

STEPS

1. Selection and Preparation of Explant

- Healthy and disease-free plant parts are selected.
- Surface sterilization using disinfectants (e.g., sodium hypochlorite) to prevent microbial contamination.

2. Initiation Phase

- Explants are placed on a nutrient medium (commonly Murashige and Skoog medium).
- Medium contains sugars, vitamins, minerals, and PGRs.
- **Objective:** Initiate cell division and callus formation or direct shoot development.

3. Multiplication Phase

- Multiple shoots or embryoids are induced by adjusting hormonal concentrations.
- Subculturing is done periodically to enhance multiplication rate.

4. Rooting Phase

- Shoots are transferred to root induction medium rich in auxins like IBA or NAA.
- Roots develop and form complete plantlets.

5. Acclimatization (Hardening)

- Plantlets are gradually introduced to external conditions.
- Initially transferred to greenhouses in controlled humidity before moving to the field.

ORGANOGENESIS

Organogenesis is a critical process in plant tissue culture

that refers to the formation of organs (shoots and roots) from undifferentiated plant cells (callus) or explants under in vitro conditions. It is a type of morphogenesis that allows the regeneration of complete plants from small tissue fragments, contributing significantly to plant biotechnology, agriculture, horticulture, and conservation efforts. This process is especially important for mass propagation of genetically uniform and disease-free plants. Organogenesis can occur either directly from explants (direct organogenesis) or indirectly via an intervening callus phase (indirect organogenesis). Hormonal balance, particularly the ratio of auxins to cytokinins, plays a key role in determining whether roots or shoots are induced.

PROCEDURE

Organogenesis in plants typically involves the following key steps:

1. Selection and Preparation of Explant

- The first step involves selecting a suitable part of the plant (explant) such as leaf, stem, root, or meristem tissue. The chosen explant must be healthy, actively growing, and genetically stable.

2. Surface Sterilization

- The explant is thoroughly sterilized using chemicals like sodium hypochlorite or ethanol to eliminate surface contaminants such as bacteria and fungi. This step ensures aseptic conditions for in vitro culture.

3. Inoculation onto Nutrient Medium

- The sterilized explant is placed onto a nutrient medium, commonly Murashige and Skoog (MS) medium, enriched with plant growth regulators (PGRs). The specific concentration of cytokinins (e.g., BAP, kinetin) and auxins (e.g., NAA, IAA) is tailored to induce shoot or root formation.

- High cytokinin:auxin ratio usually promotes shoot formation.
- High auxin:cytokinin ratio generally induces root formation.

4. Callus Formation (in Indirect Organogenesis)

- In indirect organogenesis, the explant first forms a callus (an undifferentiated mass of cells) under the influence of specific PGRs. The callus is later transferred to another medium that supports organ differentiation.

5. Organ Induction

- Under the influence of appropriate PGRs, the callus or explant starts differentiating into either shoots or roots. Multiple shoots can arise from a single explant in favorable conditions.

6. Shoot Elongation and Rooting

- Induced shoots are elongated on hormone-supplemented medium, followed by transferring to rooting medium where auxins stimulate root development.

7. Acclimatization (Hardening)

- The regenerated plantlets are moved to ex vitro conditions, where they are gradually adapted to normal environmental conditions like humidity and light. This step is vital for survival during transplantation to soil.

SOMATIC EMBRYOGENESIS

Somatic embryogenesis is a powerful and versatile technique in plant tissue culture that involves the development of embryos from somatic or non-reproductive cells, rather than from fertilized eggs (zygotes). These embryos are morphologically and developmentally similar to zygotic embryos and can give rise to entire plants. The phenomenon demonstrates the totipotent nature of plant cells, where even

fully differentiated somatic cells can be reprogrammed to initiate a complete developmental pathway. Somatic embryogenesis holds immense importance in the fields of plant biotechnology, genetic transformation, synthetic seed production, and large-scale propagation of elite cultivars. It is particularly valuable for the propagation of high-value crops, woody plants, and species that are difficult to regenerate through traditional methods.

TYPES

Somatic embryogenesis is generally categorized into two types:

1. **Direct Somatic Embryogenesis:** Embryos develop directly from explants without an intervening callus phase. This often occurs in tissues with high cellular totipotency, such as immature embryos or young leaves.
2. **Indirect Somatic Embryogenesis:** In this more common route, the explant first produces a callus—a mass of undifferentiated cells—from which somatic embryos are later induced.

PROCEDURE

The process of somatic embryogenesis generally follows these key steps:

1. **Selection and Preparation of Explant**

- The choice of explant plays a crucial role in the success of somatic embryogenesis. Explants such as immature embryos, leaf discs, hypocotyls, cotyledons, and shoot tips are commonly used. Young, actively dividing tissues are often more responsive to embryogenic induction.

2. **Sterilization of Explant**

- To maintain aseptic conditions, the explant is subjected to surface sterilization using agents like ethanol, sodium hypochlorite, or mercuric chloride, followed by

thorough rinsing with sterile distilled water.

3. Callus Induction (for Indirect Embryogenesis)

- The sterilized explant is cultured on a nutrient medium such as Murashige and Skoog (MS) medium supplemented with plant growth regulators (PGRs), particularly auxins like 2,4-Dichlorophenoxyacetic acid (2,4-D). This high-auxin medium promotes the dedifferentiation of cells and induces callus formation.

4. Embryo Induction and Development

- The callus or explant is transferred to a medium with reduced or no auxin to trigger the initiation of somatic embryos. These embryos pass through several well-defined stages resembling zygotic embryogenesis:
  A. Globular stage
  B. Heart stage
  C. Torpedo stage
  D. Cotyledonary stage

5. Embryo Maturation

- To become fully functional, somatic embryos must undergo a maturation phase. The maturation medium often includes abscisic acid (ABA) and high sucrose levels, which help in proper embryo development and desiccation tolerance.

6. Embryo Germination and Plantlet Formation

- Mature somatic embryos are transferred to germination medium containing cytokinins (e.g., BAP or kinetin) to promote shoot and root formation. These plantlets are eventually capable of autotrophic growth.

7. Acclimatization (Hardening)

- Regenerated plantlets are gradually adapted to external environmental conditions by transferring them to soil or compost in controlled environments (e.g.,

greenhouse or mist chambers). This step is essential for ensuring high survival rates during field transplantation.

APPLICATIONS OF PLANT TISSUE CULTURE

1. **Mass Propagation–** Rapid and large-scale multiplication of elite or rare plant genotypes.
2. **Genetic Engineering–** Regeneration of transgenic plants after transformation.
3. **Synthetic Seeds–** Somatic embryos can be encapsulated to produce artificial seeds.
4. **Germplasm Conservation–** Preservation of endangered species via in vitro storage.
5. **Cryopreservation–** Somatic embryos serve as excellent material for long-term cold storage.

ADVANTAGES OF PLANT TISSUE CULTURE

- **Rapid Multiplication:** Thousands of plants can be produced from a single explant in a short time.
- **Genetic Uniformity:** Produces true-to-type clones of the parent plant.
- **Disease-Free Plants:** Cultures can be cleaned of viruses and other pathogens.
- **Conservation:** Aids in preserving endangered or rare species.
- **Year-Round Production:** Not limited by seasonal changes.
- **Commercial Horticulture:** Propagation of ornamentals like orchids, lilies, and ferns.
- **Forestry:** Clonal propagation of elite tree species like eucalyptus and teak.
- **Agriculture:** Multiplication of disease-free crops like banana, potato, and sugarcane.
- **Genetic Engineering:** Used in regenerating transformed plants.

LIMITATIONS OF PLANT TISSUE CULTURE

- **Cost:** Requires expensive laboratory setup and skilled personnel.
- **Contamination Risk:** High risk of microbial contamination during culture.
- **Somaclonal Variation:** Genetic changes may occur, especially during prolonged culture.

REFERENCES

- *Desai, P., Desai, S., Rafaliya, R., Patil, G. (2022). Plant tissue culture: Somatic embryogenesis and organogenesis*. Advances in Plant Tissue Culture, *Pages 109-130. ISBN 9780323907958. https://doi.org/10.1016/B978-0-323-90795-8.00006-0*
- *Soumare, A., Diédhiou, A.G., Arora, N.K., Al-Ani, L.K.T., Ngom, M., Fall, S., Hafidi, M., Ouhdouch, Y., Kouisni, L. and Sy, M.O. (2021). Potential Role and Utilization of Plant Growth Promoting Microbes in Plant Tissue Culture*. Front. Microbiol., *12:649878. doi: 10.3389/fmicb.2021.649878*
- *Bidabadi, S. S., and Jain, S. M. (2020). Cellular, molecular, and physiological aspects of in vitro plant regeneration.* Plan. Theory, *9:702. doi: 10.3390/ plants9060702*
- *Akin-Idowu, P. E., Ibitoye, D. O., and Ademoyegun, O. T. (2009). Tissue culture as a plant production technique for horticultural crops*. Afr. J. Biotechnol., *8, 3782–3788. doi: 10.4314/ ajb.v8i16.62060*
- *Dias, J. P. T. (2019). Plant growth regulators in horticulture: practices and perspectives.* Biotecnologia Vegetal, *19, 3–14.*
- *Gupta, N., Jain, V., Rosy, M., Josepeh, Dev, S. (2020). A Review on Micropropagation Culture Method.* Asian Journal of Pharmaceutical Research and Development, *8(1):86-93. DOI: http://dx.doi.org/10.22270/ajprd.v8i1.653*
- *Bhoite, H.A. and Palshikar, G.S. (2014). Plant tissue culture: A Review.* World J. Pharma. Sci., *2:565-572*