

Lecture 7. Cultivation of Plant Cells and Tissues In Vitro: Methods of Cultivation, Plant Regeneration, and Micropropagation

Introduction

Plant cell and tissue culture is one of the fundamental technologies of modern plant biotechnology and bioengineering. The term **in vitro** means “in glass” and refers to the cultivation of plant cells, tissues, organs, or embryos outside the whole plant under controlled laboratory conditions. In vitro culture makes it possible to control the nutritional, hormonal, physical, and environmental conditions surrounding plant material and therefore provides an effective system for studying plant growth, development, differentiation, and regeneration. The technology is widely used for rapid propagation of valuable plants, production of disease-free planting material, conservation of genetic resources, plant breeding, genetic transformation, genome editing, production of secondary metabolites, and fundamental research. One of the most important biological principles underlying plant tissue culture is **totipotency**, which describes the capacity of a plant cell to contain the genetic information necessary to generate an entire plant when suitable developmental and environmental conditions are provided. Although not every isolated plant cell readily expresses this potential, appropriate culture conditions can stimulate many types of plant cells to divide, differentiate, and eventually regenerate shoots, roots, embryos, or complete plants.

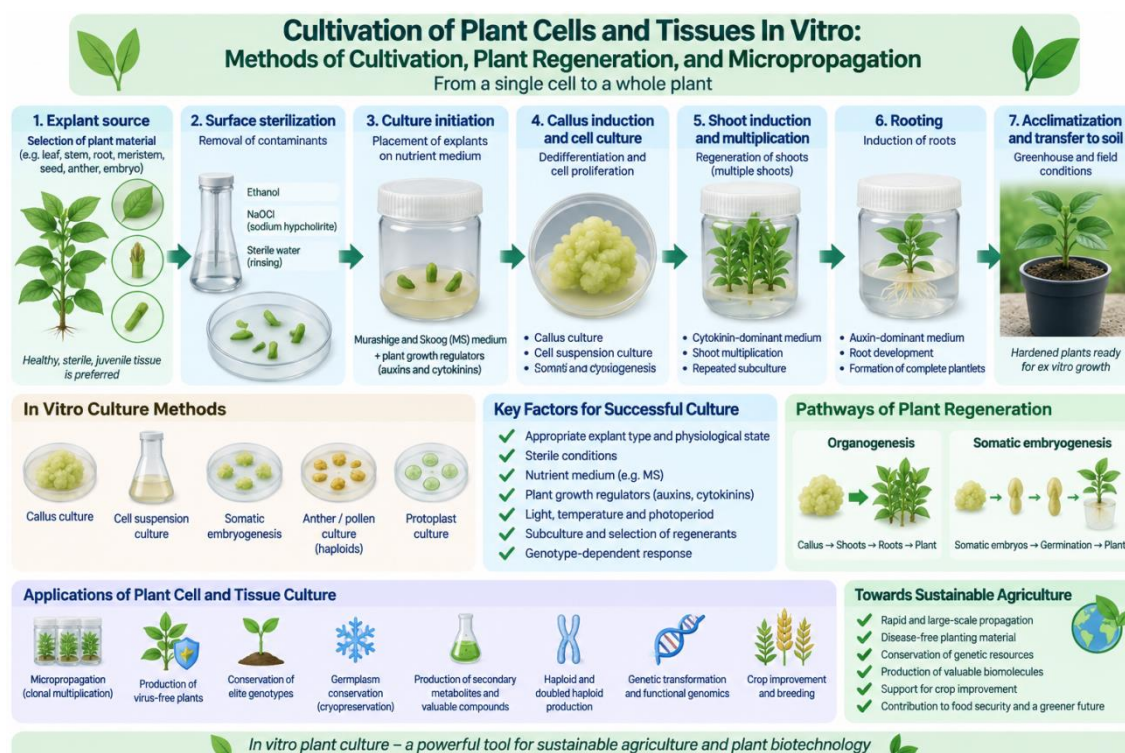


Figure 1. Graphical Abstract of Plant Cell and Tissue Culture In Vitro: Cultivation Methods, Plant Regeneration, and Micropropagation

Plant tissue culture usually begins with the selection of a small piece of plant material called an **explant**. Explants may originate from leaves, stems, roots, shoot tips, meristems, embryos, cotyledons, anthers, pollen, or other tissues. The choice of explant strongly influences the success of the culture because different tissues vary in their physiological condition, developmental stage, regenerative capacity, and response to plant growth regulators. Young and actively growing tissues are often particularly responsive to in vitro conditions. The selected plant material is introduced into a sterile culture environment containing water, mineral nutrients, a carbon source, vitamins, and appropriate plant growth regulators. Under suitable conditions, cells may begin dividing and form an unorganized mass of cells known as **callus**, or the tissue may directly produce shoots, roots, or embryo-like structures. These processes provide the biological foundation for plant regeneration and micropropagation.

Principles of Plant Cell and Tissue Culture

Successful in vitro cultivation depends on maintaining an environment that supports plant growth while preventing contamination by microorganisms. Plant tissues contain nutrients that can also support the rapid growth of bacteria and fungi, so aseptic conditions are essential. Laboratory equipment, culture vessels, nutrient media, instruments, and plant material must therefore be handled using appropriate sterile techniques. Contamination can compete with plant tissues for nutrients, produce toxic metabolites, and eventually destroy the culture. The physiological state of the donor plant is also important because healthy plant material generally provides better explants. After establishment in culture, plant cells depend strongly on the composition of the nutrient medium. One of the most widely known formulations in plant biotechnology is the **Murashige and Skoog (MS) medium**, which provides mineral nutrients required for plant growth. Culture media generally contain macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, together with micronutrients including iron, manganese, zinc, boron, copper, and molybdenum. Vitamins and an external carbon source are frequently included because small cultured tissues may not initially perform enough photosynthesis to support rapid growth.

Plant growth regulators are especially important because they influence cell division, differentiation, organ formation, and regeneration. **Auxins** and **cytokinins** are among the most important classes of growth regulators used in plant tissue culture. Their effects depend on plant species, genotype, tissue type, physiological state, concentration, and interaction with other hormones. Auxins are commonly associated with cell expansion, root development, and certain forms of callus formation, whereas cytokinins are strongly involved in cell division and shoot development. Manipulation of the hormonal environment can therefore redirect the developmental program of cultured tissues. Other plant growth regulators, including gibberellins and abscisic acid, may also be important for particular culture systems. Light intensity, photoperiod, temperature, humidity, gas exchange, and the physical characteristics of the culture medium additionally influence development. Successful tissue culture is therefore not determined by a single factor but by interactions among genotype, explant type, nutrients, hormones, and environmental conditions.

Callus and Cell Suspension Cultures

Callus culture is an important form of plant tissue culture in which differentiated plant tissues produce a proliferating mass of relatively unorganized cells. During callus formation, some cells undergo changes in developmental state and regain the capacity for active division. This process is often described in terms of **dedifferentiation**, although callus tissue itself may contain cells with different developmental characteristics. Callus cultures are valuable for studies of cell differentiation, plant regeneration, genetic transformation, selection of cellular variants, and production of certain secondary metabolites. Under appropriate conditions, callus tissue may subsequently undergo redifferentiation and produce shoots, roots, or somatic embryos. The capacity for regeneration differs greatly between plant species and even among cultivars of the same species, which makes optimization of tissue-culture conditions an important component of plant biotechnology.

Cells derived from callus can also be maintained as **cell suspension cultures**. In this system, individual cells or small groups of cells grow in a liquid nutrient medium rather than on a solid surface. Suspension cultures provide a relatively uniform biological system that can be useful for studying cell growth, metabolism, biochemical pathways, and responses to environmental factors. They can also serve as platforms for the production of plant secondary metabolites and other biologically active compounds. Because plant cells synthesize a wide range of natural products, including pigments, flavors, fragrances, antioxidants, and pharmaceutical compounds, controlled cell culture can sometimes provide an alternative production platform. However, the metabolic characteristics of cultured cells may differ from those of intact plants, so culture conditions must be optimized according to the desired biological product.

Plant Regeneration

Plant regeneration is the process through which cultured cells or tissues develop into complete plants. Two major regeneration pathways are recognized: **organogenesis** and **somatic embryogenesis**. During organogenesis, new organs such as shoots and roots develop from cultured tissue. Organogenesis may occur directly from the explant or indirectly through an intermediate callus stage. In direct organogenesis, shoots or roots arise relatively directly from the original tissue. In indirect organogenesis, the explant first forms callus, after which organs differentiate from the proliferating cells. The balance of plant growth regulators, particularly auxins and cytokinins, plays an important role in directing these developmental responses. Once shoots have formed, they can be transferred to conditions that promote root formation, producing complete plantlets that can later be adapted to external growth conditions.

Somatic embryogenesis represents a different regeneration pathway. In this process, somatic cells develop embryo-like structures capable of forming complete plants. These structures pass through developmental stages that resemble aspects of zygotic embryo development but originate from non-reproductive cells. Somatic embryogenesis is important for large-scale propagation, plant breeding, genetic transformation, and research into developmental biology. It can also provide a useful system for the production of artificial or synthetic seeds when somatic embryos are encapsulated in suitable materials. Both organogenesis and somatic embryogenesis demonstrate the remarkable developmental plasticity of plant cells and provide essential tools for modern plant biotechnology.

Micropropagation

Micropropagation is the rapid multiplication of plants under in vitro conditions. It is especially valuable for plants that reproduce slowly through conventional methods, plants with valuable genetic characteristics, vegetatively propagated crops, ornamental plants, horticultural species, forest plants, and certain endangered or rare species. A major advantage of micropropagation is the possibility of producing large numbers of plants from a relatively small amount of starting material while maintaining selected characteristics of the original genotype. The process is based on repeated multiplication of shoots or other regenerative structures followed by rooting and adaptation of regenerated plants to external conditions.

Micropropagation can be understood as a sequence of developmental stages. First, appropriate donor plants and explants are selected and cultures are established under aseptic conditions. The next major phase is multiplication, during which the number of shoots or regenerative structures is increased through repeated culture. Individual shoots are then encouraged to develop roots and become complete plantlets. The final stage is **acclimatization**, sometimes called hardening, during which plants produced under controlled in vitro conditions gradually adapt to external conditions. This stage is important because plantlets grown inside culture vessels develop under high humidity, relatively stable temperatures, controlled light, and a nutrient-rich environment. Their leaves, stomatal function, roots, and protective surface structures may therefore initially be poorly adapted to greenhouse or field environments. Gradual acclimatization allows regenerated plants to develop more effective physiological control over water balance and photosynthesis before transfer to normal cultivation conditions.

Meristem Culture and Production of Healthy Planting Material

Meristem culture is an important specialized application of plant tissue culture. The shoot apical meristem consists of actively dividing cells located at the growing tip of a shoot. Cultivation of meristematic tissue can be useful for obtaining healthy planting material, particularly in vegetatively propagated crops. In some plant–pathogen systems, meristem culture is used as part of strategies for obtaining plants free from particular systemic pathogens. This has important applications in crops such as potato, fruit plants, ornamentals, and other clonally propagated species. Healthy starting material can then be multiplied through micropropagation to generate large numbers of plants with improved phytosanitary status.

Plant tissue culture can therefore contribute directly to agricultural production by providing standardized planting material. Conventional vegetative propagation may gradually spread pathogens from one generation to another because the same biological material is repeatedly reproduced. Tissue-culture technologies, combined with appropriate diagnostic methods, can help establish clean propagation systems. Molecular methods may subsequently be used to confirm the identity of plant material or evaluate the presence of particular pathogens.

Anther, Pollen, and Embryo Culture

Plant tissue culture is also valuable in breeding programs. **Anther and pollen cultures** can be used in certain species to obtain haploid plants. Haploid cells contain a single set of chromosomes, and chromosome doubling can produce completely homozygous lines in a relatively short period. Such **doubled haploid** plants are extremely useful for genetic analysis and breeding because homozygous lines can otherwise require several generations of self-pollination. The efficiency of haploid production varies substantially among species and genotypes, but when an effective system is available it can significantly accelerate breeding programs.

Embryo culture is another important technique. Embryos can be isolated and cultivated under controlled conditions when normal seed development or germination is difficult. In plant breeding, embryo culture may be used as part of **embryo rescue**, particularly when hybridization between genetically distant parents produces embryos that cannot complete normal development within the seed. Cultivation in vitro can sometimes allow these embryos to survive and generate plants, expanding the range of genetic diversity available to breeders.

Protoplast Culture and Somatic Hybridization

A **protoplast** is a plant cell from which the cell wall has been removed. Without the cell wall, the plasma membrane becomes directly accessible, making protoplasts useful for studies of cell biology, genetic transformation, genome editing, and cell fusion. Under suitable conditions, some protoplasts can rebuild their cell walls, divide, form colonies, and eventually regenerate plants. However, regeneration from protoplasts is highly species-dependent and remains difficult in many crops.

Protoplasts from different plant cells can also be fused to produce **somatic hybrids**. Somatic hybridization can combine cellular material from plants that may be difficult to cross through conventional sexual reproduction. This approach has been used experimentally to transfer useful characteristics between related plant materials and to study interactions between nuclear and cytoplasmic genomes. Although modern molecular and genome-editing technologies have provided additional approaches to crop improvement, protoplast systems remain valuable for fundamental research and for the delivery of genome-editing components.

Genetic Transformation, Genome Editing, and Tissue Culture

Plant cell and tissue culture is closely connected with genetic engineering. During many transformation procedures, genetic material is introduced into individual cells or small groups of cells. These modified cells must then be regenerated into complete plants if the genetic modification is to be studied at the whole-organism level. Consequently, efficient plant regeneration is often one of the most important requirements for successful genetic transformation. *Agrobacterium*-mediated transformation, particle bombardment, and protoplast-based delivery systems are frequently combined with tissue culture to generate transformed plants.

The same principle applies to genome editing. CRISPR/Cas and related technologies can create targeted genomic modifications, but the edited cells must often be regenerated into

plants. Some economically important crops remain difficult to edit not because DNA targeting itself is impossible but because transformation and regeneration are inefficient or strongly genotype-dependent. Improvement of tissue-culture systems is therefore an important component of modern genome-editing research. Advances that increase regeneration efficiency or reduce genotype dependence can significantly expand the practical application of genome engineering in agriculture.

Genetic Stability and Somaclonal Variation

One objective of micropropagation is frequently the production of genetically uniform plants. However, prolonged cultivation under in vitro conditions can sometimes produce genetic or epigenetic variation known as **somaclonal variation**. Such changes may arise during cell division, callus formation, regeneration, or extended culture. Somaclonal variation can be undesirable when the objective is to produce genetically identical clones because regenerated plants may differ from the original donor plant in morphology, development, fertility, or other characteristics. Therefore, maintaining genetic fidelity is important in commercial micropropagation and conservation programs.

At the same time, somaclonal variation can occasionally provide useful new genetic diversity for plant breeding. Plants showing potentially valuable characteristics can be selected and evaluated further. Molecular markers, cytogenetic analysis, sequencing, and phenotypic assessment can be used to investigate genetic stability. The biological significance of somaclonal variation therefore depends on the purpose of the culture: variation is generally minimized during clonal propagation but may sometimes be explored as a source of new traits in breeding research.

Germplasm Conservation and Cryopreservation

Plant tissue culture also plays an important role in the conservation of genetic resources. Valuable plant genotypes can be maintained as in vitro collections when conservation through seeds is difficult or inappropriate. This is particularly relevant for vegetatively propagated crops and plants producing seeds that cannot be stored easily for long periods. Slow-growth culture techniques can reduce the frequency with which cultures must be transferred to fresh media, allowing plant material to be maintained for extended periods.

For longer-term conservation, **cryopreservation** can be used. Cryopreservation involves storage of biological material at extremely low temperatures, greatly reducing metabolic activity and allowing genetic resources to be preserved for long periods. Shoot tips, embryos, cell cultures, or other suitable plant materials may be conserved depending on the species and objective. These approaches are important for maintaining agricultural biodiversity, protecting valuable breeding material, and supporting future crop-improvement programs.

Applications in Agriculture and Biotechnology

Plant cell and tissue culture has numerous applications in modern agriculture. Micropropagation enables rapid multiplication of valuable cultivars and can support the production of uniform planting material. Meristem-based techniques contribute to programs for generating healthy propagation stocks. Tissue culture facilitates the conservation and

distribution of valuable genetic resources and supports breeding through haploid production, embryo rescue, and regeneration of selected genotypes. It is also essential for many genetic transformation and genome-editing programs. Plant cell cultures can be used to investigate metabolic pathways and produce secondary metabolites, pigments, flavors, pharmaceutical compounds, and other valuable biological products. In forestry and horticulture, tissue culture can help multiply elite genotypes that may be difficult to propagate through seeds or conventional vegetative techniques.

In vitro technologies also contribute to the conservation of rare and endangered plants by allowing valuable genetic material to be multiplied and maintained under controlled conditions. However, laboratory propagation should be combined with appropriate conservation strategies because successful preservation of biodiversity requires protection of natural populations and ecosystems as well as ex situ conservation. Tissue culture is therefore best viewed as one component of a broader strategy for sustainable management of plant genetic resources.

Advantages and Limitations

The main advantages of plant tissue culture include rapid propagation, production of large numbers of plants from limited starting material, year-round propagation independent of normal growing seasons, conservation of valuable genotypes, support for pathogen-management programs, and compatibility with modern genetic technologies. Controlled culture conditions also make it possible to investigate plant development and metabolism in ways that are difficult to achieve in whole plants under field conditions.

Nevertheless, tissue culture has several limitations. Different plant species and genotypes can respond very differently to identical culture conditions. Some plants regenerate easily, while others remain highly recalcitrant. Contamination can cause significant losses, and prolonged culture may increase the risk of unwanted genetic or epigenetic variation. Specialized facilities, trained personnel, and careful quality control may be required for large-scale commercial production. The cost of labor, energy, culture media, and acclimatization can also influence economic feasibility. Research therefore continues to focus on improving culture efficiency, automation, genetic stability, and regeneration capacity.

Future Perspectives

The future of plant tissue culture is closely connected with genome editing, synthetic biology, automated cultivation, advanced imaging, bioinformatics, and artificial intelligence. Automated systems may reduce manual handling and increase the scale of micropropagation, while improved imaging and machine-learning technologies may assist in monitoring tissue growth and identifying healthy cultures. New knowledge about plant developmental regulators may help overcome regeneration difficulties in recalcitrant species. Integration of tissue culture with CRISPR/Cas genome editing could make it possible to generate improved plant varieties more efficiently, while advances in cryobiology will strengthen the long-term conservation of plant genetic resources.

Modern plant biotechnology is therefore moving toward an integrated system in which **genomics identifies important genes, genome editing modifies them, tissue culture regenerates modified cells into plants, molecular analysis confirms the changes, and**

phenotyping determines their agricultural value. In this system, plant cell and tissue culture remains a central technology connecting molecular modification with the production of complete plants.

Conclusion

Cultivation of plant cells and tissues in vitro is a fundamental technology of plant biotechnology based on the developmental plasticity and regenerative potential of plant cells. By providing controlled nutritional, hormonal, and environmental conditions, researchers can maintain cells and tissues, induce callus formation, regenerate shoots and roots, produce somatic embryos, and generate complete plants. Major applications include **micropropagation, plant regeneration, production of healthy planting material, haploid and doubled-haploid production, embryo culture, protoplast research, genetic transformation, genome editing, germplasm conservation, cryopreservation, and production of valuable metabolites.** Micropropagation is particularly important because it allows rapid multiplication of selected plant genotypes, while organogenesis and somatic embryogenesis provide essential pathways for regeneration. Despite challenges associated with contamination, genotype dependence, regeneration efficiency, cost, and somaclonal variation, in vitro culture continues to play a central role in modern plant science. Combined with genomics, genome editing, protein bioengineering, and advanced breeding technologies, plant tissue culture provides an essential platform for crop improvement, conservation of genetic resources, sustainable agriculture, and future development of plant biotechnology.