Chapter-4

Haploid Culture Techniques : Callus Culture, Anther Culture and Ovule Culture.

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ABSTRACT

In order to create haploid plants, haploid cells (which have only one set of chromosomes) are grown in vitro using haploid culture techniques. By avoiding the usual fertilization process, these methods frequently use anther or pollen culture, in which the tissues of male gametophytes are stimulated to create embryos. These techniques are important because they speed up plant breeding efforts and make genetic research easier by enabling the quick creation of homozygous lines through chromosome doubling. Additionally, haploid culture makes it possible to produce plants with unique features and examine gene expression in a reduced genomic setting.

KEYWORDS: Tissue Culture, Callus, Haploid plants, Anther culture, Ovule, Plant Biotechnology.

INTRODUCTION

In vitro plant tissue culture is a technique that allows for the growth and maintenance of plant cells, tissues, or organs under sterile conditions on a nutrient medium. It relies on the principle of totipotency, which means that a single plant cell can regenerate into an entire plant. With this method, a small piece of tissue (like a leaf, stem, or root) can be used to quickly produce genetically identical plants (clones). Tissue culture is extensively employed in agriculture, horticulture, and biotechnology for various purposes, including mass propagation, conservation of rare or endangered species, generation of disease-free plants, and genetic engineering. The procedure consists of multiple phases: choosing explants, surface sterilization, callus induction, development of shoots and roots, and plantlet acclimatization to the natural environment.

Plant tissue culture plays a major role in agriculture, providing novel methods for enhancing crops and promoting sustainability in farming practices. It is primarily used for the mass propagation of high-yielding, disease-free, and genetically uniform plants in a short time, which is particularly valuable for crops such as banana, potato, and sugarcane. It also aids in the preservation of elite and endangered plant species, contributing to the maintenance of genetic diversity. Moreover, tissue culture allows for the creation of planting material that is free from pathogens, which decreases the dependence on chemical treatments and enhances crop health. It is essential for genetic engineering, making it possible to create transgenic crops with enhanced characteristics like resistance to pests, tolerance of drought, and improved nutritional value. Furthermore, micropropagation through tissue culture supports the rapid introduction of new varieties to farmers, accelerating agricultural productivity and resilience in the face of climate change and evolving plant diseases.

CALLUS CULTURE

In the field of plant tissue culture, the method of callus culture involves generating callus (unorganized, undifferentiated cell masses) from plant explants like leaves, stems, or roots in a sterile environment. Usually, this process entails putting the explant on a nutrient medium enriched with plant growth regulators, especially auxins such as 2,4-D or NAA, and occasionally cytokinins. Calluses (Calli) provide totipotent cells that can later be prompted to develop into shoots, roots, or entire plants via somatic embryogenesis or organogenesis. Callus culture is extensively employed for plant regeneration, genetic transformation, mutagenesis, and secondary metabolite production. It is also useful for examining plant development, gene expression, and stress responses in research. This method plays a crucial role in plant biotechnology and crop improvement programs, offering a foundation for advanced applications such as synthetic seed production and genetic engineering.

HISTORY

- **Henri-Louis Duhamel du Monceau (1756):** Pioneered experiments on wound healing in plants, observing callus formation on wounded elm trees, marking the initial observation of callus in live plants.
- **Haberlandt (1902):** In 1902, Gottlieb Haberlandt was the first to establish plant callus culture. He was the first to develop the idea of cell culture in plants, establishing the basis for today's plant tissue culture methods.
- **P. White, E. Gautheret, and P. Nobécourt (1939):** Independently reported successful callus induction in vitro, using different plant species and media. White induced callus from tumor-developing tissues of Nicotiana glauca, Gautheret and Nobécourt established

continuous callus cultures of carrot using auxin hormone additions.

- **Nobécourt (1937-1939):** Established the first callus culture capable of continuous growth on a semisolid agar medium, using a *Daucus carota* tap root explant.
- **J. Van Overbeck, M.E. Conklin, and A.F. Blakeslee (1941):** Reported the importance of coconut milk in callus culture, providing essential nutrients for plant cell growth.
- **S.M. Caplin and F.C. Steward (1948):** First successfully grew differentiated, non-cambial cells from *Daucus carota* using coconut milk in the medium, later demonstrating cell division induction with synthetic auxin.
- **1960s onwards:** Research focused on understanding cell behavior, metabolism, and morphogenesis in callus cultures, leading to applications in plant pathogen eradication, germplasm storage, and clonal propagation.

CALLUS TYPES

Based on parameters such as texture, color, and cellular characteristics, plant callus can be classified into several types, which often indicate the tissue's physiological state and regenerative potential. The common types of calluses are as follow:

- **Friable:** Marked by cells that are loosely grouped together, can easily break apart, and often have a white or creamy yellow appearance.
- **Compact:** Features tightly packed cells, with a green and robust appearance.
- **Regenerating**: Calli capable of developing into complete plants via organogenesis (formation of shoots and roots) or embryogenesis (development of somatic embryos). They can be further divided into shoot calli, root calli, or embryogenic calli.

- Non-Regenerating: Calli utilized for cell suspension cultures and metabolite production that do not generate organs.

PROTOCOL FOR CALLUS CULTURE

Callus culture, a widely used method in plant tissue culture, involves inducing and sustaining undifferentiated cell masses (callus) from plant explants in a sterile environment. Below is a general protocol for callus culture:

Explant Selection and Preparation:

- Select an explant (a segment of plant tissue) that has a good chance of forming callus.
- Surface-sterilize the explant to eliminate any microbial contamination. Common methods involve the use of ethanol, bleach, or mercury bichloride.
- Prepare the explant by cutting it into smaller segments and handling it correctly to prevent contamination.

Medium Preparation:

- Prepare a sterile nutrient medium that supplies the essential nutrients and minerals for callus growth.
- Well-known media comprise Murashige and Skoog (MS) medium, White's medium, and woody plant medium.
- To induce callus formation, incorporate plant growth regulators into the medium, including auxins (such as 2,4-D or NAA) and cytokinins (like BA or kinetin).

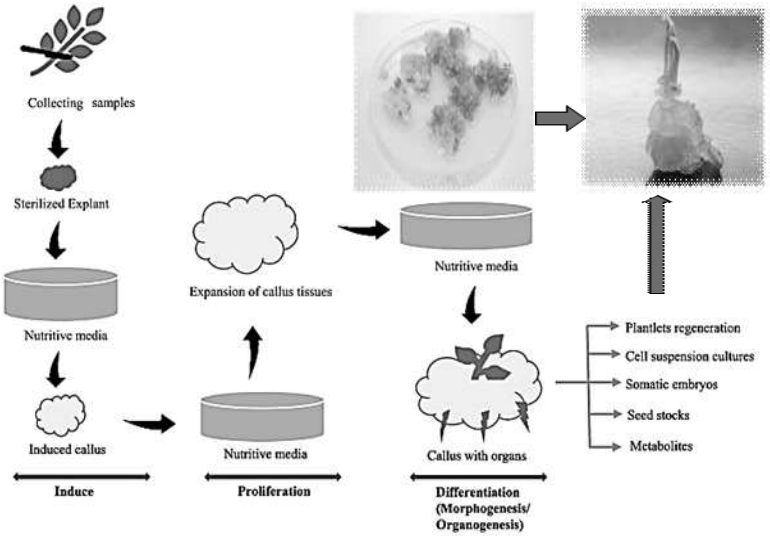

Figure 1. The picture represents various stages of callus culture.

Inoculation and Incubation:

- Disinfect the explant and place it on the prepared medium.
- Place the culture in a controlled environment for incubation, ensuring proper conditions for temperature (typically 25-28°C), light (16 hours of light and 8 hours of darkness), and humidity.

Callus Proliferation and Maintenance:

- Observe the growth of the callus and modify conditions as necessary.
- To ensure healthy growth and avoid nutrient depletion, periodically subculture the callus.

APPLICATION OF CALLUS CULTURE IN AGRICULTURE

Callus culture is significantly useful in agriculture, especially for plant breeding and enhancing crops. A major application

of this technique is in plant regeneration via tissue culture methods, which allows for the swift propagation of genetically uniform plants that exhibit beneficial characteristics like enhanced yield, disease resistance, or drought tolerance. It serves as a crucial step in genetic transformation, allowing for the introduction of foreign genes into callus tissue, which can then be regenerated into complete transgenic plants. Moreover, callus culture fosters somaclonal variation, which provides a genetic diversity source that can be utilized to create new cultivars. In crop biotechnology, it facilitates the mass production of secondary metabolites, thereby increasing the value of medicinal and aromatic plants.

LIMITATIONS OF CALLUS CULTURE

- **Genetic Instability:** Somaclonal variation can arise from callus cultures, leading to undesirable genetic mutations that might impact the uniformity and stability of regenerated plants.
- **Low Regeneration Efficiency:** Certain plant species or callus types do not regenerate efficiently into whole plants; some may remain undifferentiated or necrotic.
- **High Cost and Labor Intensive:** Ensuring sterility, preparing culture media, and ongoing subculturing demand specialized tools, considerable time investment, and trained individuals.
- **Hormone Sensitivity:** The effectiveness of callus induction and regeneration is largely determined by the exact equilibrium of plant growth regulators, a factor that can differ greatly among species and even among explants.
- **Tissue Browning and Necrosis:** The oxidation of phenolic compounds in certain explants can result in browning, which may cause callus death and diminish viability.

ANDROGENESIS

In plant tissue culture, androgenesis refers to the process whereby male reproductive cells (microspores or immature anther cells) develop into haploid plants without the need for fertilization. This method is essentially for anther and microspore culture, and it is commonly employed in genetic research and plant breeding. In androgenesis, the microspore is diverted from its typical route of developing into sperm cells and instead follows a developmental trajectory that results in the formation of a complete plant. Haploid plants produced in this way have only one set of chromosomes, which makes them very useful for generating homozygous lines after chromosome doubling. This speeds up the breeding process and aids in creating new crop varieties with desirable characteristics like disease resistance, stress tolerance, and enhanced productivity.

POLLEN CULTURE

Pollen culture, or androgenesis, is a method of plant tissue culture that generates haploid plants from immature pollen grains (microspores) in sterile conditions. The success of anther culture can be affected by factors like the plant species, genotype, and so on. Haploid plants, which contain only one set of chromosomes, make it possible for breeders to quickly generate homozygous lines after chromosome doubling. This is why anther culture is commonly employed in plant breeding programs. This method is essential for plant breeding as it allows for the creation of homozygous lines through haploid chromosome doubling, which greatly shortens the time needed to attain genetic uniformity in comparison with conventional methods. Pollen culture is crucial for producing doubled haploids (DHs), which are essential in the creation of stable, high-yield, disease resistance, and stress tolerance crop varieties.

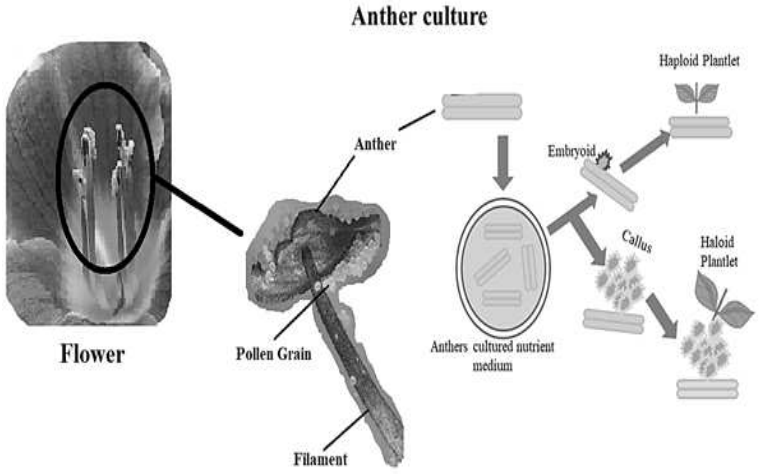

Figure 2. Haploid plant production by using anther culture technique.

Additionally, it enables the examination of gene expression and mutation at the haploid level, which can be beneficial for functional genomics. Widely applied in crops such as rice, wheat, and barley, pollen culture contributes to accelerated crop improvement programs, making it a powerful tool in modern agriculture and plant biotechnology.

GYNOGENESIS

In plants, gynogenesis is a type of asexual reproduction in which an embryo forms from the female gamete (egg cell) without any genetic input from the male parent. Embryo development can be triggered without fertilization by using irradiated or genetically inactive pollen, which can artificially induce this process, often through in vitro techniques. The plants that result are haploid, containing solely the maternal chromosome set, which can later be doubled to generate homozygous diploids. Gynogenesis is especially beneficial in plant breeding programs, as it allows for the quick generation

of pure lines and accelerates the creation of new cultivars. It has been effectively used in various crop species, such as onion, wheat, and sugar beet.

OVULE CULTURE

Ovule culture, a technique in plant tissue culture, involves growing ovules under sterile in vitro conditions to aid embryo development, particularly when normal fertilization or seed development is hindered. This approach is especially useful for surmounting obstacles in interspecific and intergeneric hybridization, where fertilization can take place but embryo loss occurs prior to seed development. Ovule culture is a crucial component of embryo rescue methods, as it enables the preservation of embryos and their growth into healthy plants by cultivating ovules at suitable points in their development. It also finds application in haploid production through the culturing of unfertilized ovules, as well as in genetic research related to early embryo development and seed physiology. Ovule culture plays a significant role in plant breeding and genetic enhancement programs by facilitating the recovery of hybrids that would otherwise be lost, thereby broadening the genetic diversity available for crop improvement.

APPLICATION OF HAPLOID CULTURE

Haploid culture is of great importance for agriculture, especially in the areas of plant breeding and crop enhancement. Breeders can rapidly create homozygous diploid lines through chromosome doubling by producing haploid plants that contain only one set of chromosomes. This fosters the creation of genetically uniform and stable pure lines, which is crucial for hybrid seed production and trait fixation. Haploid culture is particularly effective for reducing breeding cycles, increasing selection efficiency, and recognizing advantageous genetic characteristics like disease resistance, drought tolerance, and yield improvement. It has been extensively used in crops such

as rice, wheat, maize, and barley, aiding in the creation of enhanced varieties that fulfil the requirements of contemporary agriculture.

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