

## Chapter-2

### **Plant Cell Suspension Culture: Method, Types, Advantages and Limitations.**

**Soumyajit Nayak<sup>1</sup>, Harekrushna Swain<sup>1</sup>, Kiran Ku. Bindhani<sup>1</sup>, and Dr. Sanjay Ku. Pattnaik<sup>2\*</sup>**

*<sup>1</sup>Department of Biotechnology, NIIS Institute of Information Science and Management, Bhubaneswar, Odisha, India.*

*<sup>2\*</sup>Department of Botany, Mahendra Institute of Management and Technical Studies, Pitapali, Odisha, India.  
[spattnaik1980@gmail.com](mailto:spattnaik1980@gmail.com)*

#### **ABSTRACT**

Cell suspension culture refers to the process of single cells growing more quickly in a liquid media. An orbital shaker is used to continuously stir the liquid medium. Scientists utilize this culturing method to investigate the growth and development of cells. Additionally, several companies use tissue culture to extract specific components from plant cells. The medium's agitation also applies a slight pressure to the tissue, causing it to fragment into single cells and smaller cell fragments. The consistent mobility and dispersion of cells throughout the media are preserved by this agitation. As a result, it is a crucial step in cell suspension culture.

**KEYWORDS:** Biotechnology, Suspension Culture, Bioreactor, Tissue Culture, Plant Products, Secondary metabolites.

#### **INTRODUCTION**

Suspension cultures are typically started by moving pieces of undifferentiated callus to a liquid medium, which is stirred during the culture period. Another method of initiating these

cultures is to inoculate a liquid medium with an explant of differentiated plant material, such as fragments of cotyledon or hypocotyls; however, this method results in longer culture times. A friable callus yields an excellent suspension culture with a high proportion of single cells and tiny cell clusters. A higher auxin:cytokinin ratio can occasionally result in a more friable culture, which improves suspension culture. Periodic subculturing is also necessary for a successful suspension culture. Subculturing is always carried out when the medium's cell density is at its highest. Soon after inoculation, the culture's growth exhibits an initial lag phase before any indication of cell division. An exponential phase follows, during which the number of cells increases exponentially. A linear phase follows shortly after, during which the cell population's growth rate continuously declines. Cell division eventually ceases, and the maximum cell density is reached. This represents the stationary phase.

An alternative method is offered by plant cell culture, which could be appealing in some situations, such as when the source plant is hard to grow, takes a long time to grow, or produces few metabolites; when chemical synthesis has not been accomplished, or when it is technically challenging. The cell culture's metabolite yield may be noticeably higher than that of the parent plant. As a result, this approach allows for the controlled and repeatable production of the metabolite regardless of climate or location.

## **CELL SUSPENSION CULTURE**

In plant biotechnology, plant cell suspension cultures are a common method for investigating a variety of phenomena without having to deal with the structural complexity of the plant organism itself. The high pace of cell development, the enormous amount of material available, the homogeneity of an *in vitro* cell population, and the high reproducibility of circumstances make suspension-cultured cells an excellent

choice for analyzing intricate physiological processes at the cellular and molecular levels. Furthermore, the generation of high-value secondary metabolites and other compounds of commercial relevance can be facilitated by plant cell cultures.

### **PHASES OF SUSPENSION CULTURE**

Selecting a strong parent material, optimizing the surface sterilization process, inducing, maintaining, and mass-propagating the callus culture in petri dishes, initiating, homogenizing, maintaining, and mass-propagating the suspension culture in shake flasks and bioreactors, and finally banking the suspension production cell line are the key phases in the entire procedure of suspension culture. While a callus culture can be started using any section of a plant, it's crucial to choose the parent plant and organ type that have the appropriate quantity and quality of the bioactive component or compounds.

Plant species, development stage, location, and organ type (also known as explant) all have a significant impact on the amount and quality of the bioactive molecule or compounds of interest. In order to create a high-performing callus culture that is friable, develops and produces effectively, and is stable, growth regulators (auxins and cytokinins) that are added to the culture medium must also be considered. Secondary metabolite synthesis and callus growth and morphology are influenced by the growth regulators' type and concentration. Following the selection of callus cell lines, a suspension cell culture is produced.

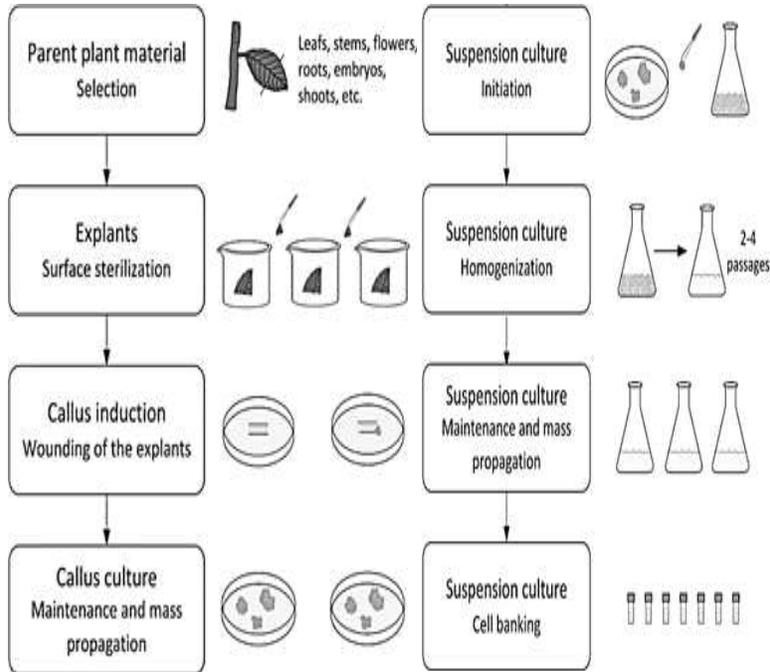


Figure 1: Standard procedure for DDC-based plant cell suspension culture (Eibl et al., 2018).

## TYPES OF CELL SUSPENSION CULTURES

In a wide prospective, cell suspension cultures can be classified into two types, such as Batch cultures and Continuous cultures.

### Batch cultures

Under the right circumstances, we can cultivate cells in a set quantity of culture medium using a closed system culture. To carry out this kind of cell suspension, flasks with a volume of up to 250 mL are utilized. For later flasks in suspension, the cells from the initial flask can serve as your inoculum. For each following subculture, a small aliquot is taken from the initial suspension and transferred to a new medium. The fact

that the cells grow to a point and then stop growing is a significant disadvantage of batch cultures. Both the quantity and size of cells stay unchanged during this stationary phase.

### **Constant cultures**

In this type of cell suspension culture, we can maintain a consistent phase of cell growth. In this location, new medium is constantly brought in while the leftover nutrients and metabolic waste products are continuously removed from the medium. Therefore, by employing this technique, you can circumvent the media's detoxification and thereby get around batch cultures' disadvantage.

### **ADVANTAGES**

- **Rapid transformation:** Transformed cell lines can be obtained and verified in a shorter timeframe than with whole plants.
- **Controlled environment:** Suspension cultures provide precise and sterile conditions, making them ideal for producing high-value recombinant proteins for clinical applications.
- **Simplified protein purification:** Depending on the expression construct employed, proteins can be readily extracted from cells or the culture medium.
- **Suitable for certain compounds:** Suspension cultures are well-suited for producing certain compounds that are not easily produced in other plant cell culture systems, like hairy roots or shooty teratomas.
- **Genetic manipulation and mutagenesis:** Suspension cultures facilitate in vitro studies for genetic manipulation, mutant initiation, and protoplast production.
- **Bioprinting and cosmetics:** They are also used in bioprinting and cosmetics industries.

## LIMITATIONS

- **High scaling-up costs:** Scaling up production in large fermentors requires significant investment and specialized personnel, making it expensive.
- **Limited productivity:** Recombinant protein yields can decrease during the late stationary phase due to increased proteolytic activity, limiting overall productivity.
- **Limited cell line diversity:** The system is primarily effective with a few well-characterized cell lines, like tobacco, rice, or Arabidopsis.
- **Decreasing productivity over time:** Suspension cultures can experience a decline in productivity and slow growth over time.
- **Clumping and stickiness:** Plant cells in suspension cultures often form clumps or become sticky, requiring modifications to the culture medium or enzymatic treatments to obtain free cells.
- **Potential for contamination:** While sterile conditions are maintained, contamination risks can still be a concern, especially during large-scale production.

## SUSPENSION CULTURE IN CROP IMPROVEMENT

Suspension cultures are especially useful in crop improvement for generating somaclonal variations, selecting for favourable characteristics like disease resistance or drought tolerance, and enabling genetic transformation. These cultures facilitate the mass production of secondary metabolites and can act as a basis for creating genetically modified crops with improved traits. In general, suspension culture is a significant resource for the progress of contemporary plant biotechnology and crop improvement initiatives.

## **BIOREACTOR**

A bioreactor is a regulated space made to facilitate the development, maintenance, and control of living things (cells or microorganisms) under certain, ideal circumstances. It is frequently used to create biological products, carry out experiments, or investigate biological processes in a variety of disciplines, such as biotechnology, medicines, and research. Bioreactors maximize the growth and synthesis of desired biological substances by controlling parameters including temperature, pH, oxygen levels, and feed delivery. Recent developments in plant cell culturing in bioreactors include 3D culture methods that imitate natural conditions, microcarrier optimization for improved growth, and genetic engineering for metabolite production.

### **Airlift bioreactors**

Airlift bioreactors, a subtype of pneumatically agitated bioreactor, utilize gas sparging and fluid circulation to establish a continuous flow of nutrients and oxygen while ensuring temperature uniformity, all without the use of mechanical agitation. Alkaloids are produced from suspension cultures of *Berberis wilsoniae*, with the formation of phenolic alkaloids being contingent on the level of dissolved oxygen.

### **Stirred-tank reactors**

Continuous Stirred-tank bioreactors (CSTR) are often employed in suspension cultures, providing effective mixing via mechanical agitation, which fosters even nutrient distribution and gas exchange. They are versatile enough for both research and large-scale production, with features such as controlled temperature, pH, and dissolved oxygen levels promoting cell growth and metabolite production. Because of their scalability, stirred-tank bioreactors serve as the workhorse in industrial environments where reproducibility and yield optimization are essential.

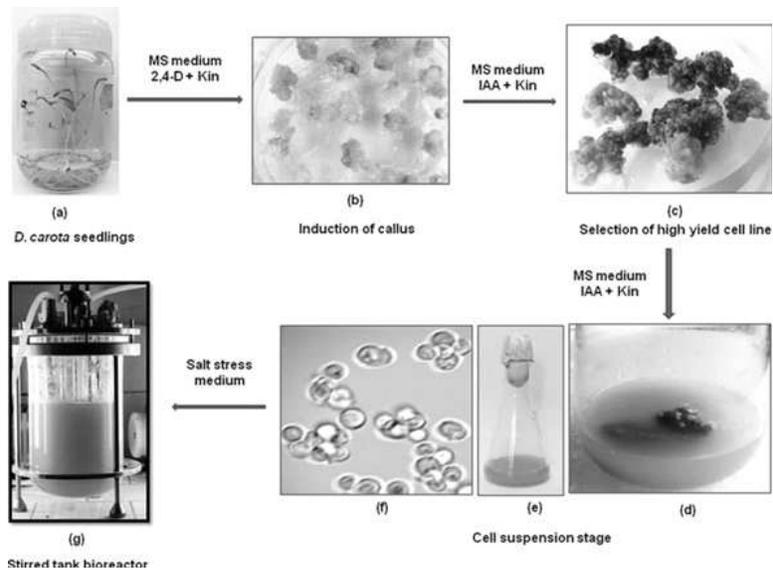


Figure 2. cell suspension culture of *D. carota*. (a) In vitro propagated, (b) callus induction on MS medium with 2,4-D and Kin, (c) anthocyanin accumulation in callus culture, (d, e) suspension culture grown in the shake flask, (f) confocal microscopy picture of anthocyanin containing cells, and (g) culture grown in the bioreactor with salt stress medium (Kirti et al., 2021).

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