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A REVIEW: ESTABLISHMENT AND APPLICATIONS OF STARCHY CROPS CELL SUSPENSION CULTURES

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ABSTRACT: Starchy crops are crops that contain starch which is the main source of dietary energy for the world's population. Starchy crops include rice, sweet potato, cassava, soybean, corn, wheat and more. Plant tissue culture offers many advantages including the regeneration of whole plants from plant cells that have been genetically modified, the production of medicinal compounds and a convenient tool for molecular levels studies. The purpose of this paper is to review the latest progress in cell suspension culture for starchy crops. Different types of starchy crops require different parameters for the optimum growth rate which depends on the purpose of establishing them. The main parameters that affect the growth of cell suspension culture are the type of media used and growth conditions such as inoculum size and agitation rate. The procedures for establishing cell suspension culture are generally similar for all types of plants. As of now, reports on cell suspension cultures for starchy crops focus more on the regeneration of plants and the transformation of plant cells for enhancing plant traits. In conclusion, the culture parameters for starchy crop cell suspension growth differed based on its application and purpose.

KEYWORDS: *starchy crops, cell suspension culture, culture parameters*

1. INTRODUCTION

Starch, which is found in starchy plants, provides most of the world's population with dietary energy. Starch is the biggest compound of carbohydrate-rich food and carbohydrate is known as an important food component. Starchy crops include rice, sweet potato, cassava, soybean, corn, wheat and more [1]. Food and industrial economies are mostly based on starch, but due to the rising population and a lack of fertile land, the food situation in the majority of tropical developing nations is deteriorating faster [2]. This resulted in high demand for starch in the food, medicinal, and industrial sectors.

Alternatively, biotechnological studies have been carried out to understand the fundamental processes in starchy plants such as in vitro experimental studies by using callus and cell suspension culture [3]. Plant cell cultures offer a promising option that is less

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expensive, more scalable, needs less infrastructure investment upfront, permits post-translational modification, and is stable in storage [2]. It also can regenerate complete plants from plant cells that have undergone genetic modification, manufacture medicinal compounds, and be an effective tool for molecular-level study.

Cell suspension cultures (CSC) are helpful experimental methods in the study of plant sub-proteome composition, including both intracellular and extracellular sub-proteomes, as well as differential gene expression in response to both biotic and abiotic stress factors [3]. CSC is defined as a multiplication or division of single cells or cell aggregates when the liquid medium is agitated. This CSC starts from the subculture of friable callus. A successful callus formation will be used to generate the CSC by adding the callus into the liquid medium. It is required to optimize the growth, maintenance and production of liquid media to produce a higher number of natural products [4]. Optimized conditions of culture medium environment can give the integrity and stability of the specific production of natural products. This review aims to evaluate the most recent developments related to the cell suspension cultures of starchy crops.

2. APPLICATIONS OF STARCHY CROPS

Starchy crops have many applications in food, industrial and pharmaceutical productions [5]. Table 1 shows the applications of starch from different starchy crops.

Table 1: Applications of starch in various industries

Starchy crops	Applications	References
Rice starch	Orodispersible (ODF) film former	[6]
Cassava starch	• Thickening agent • Textiles • Paper • Sweeteners	[7]
Purple sweet potato	• Food like noodles, breads, jams • Cosmetics like lipstick, rouge and shampoo • Pharmaceutical–coloured tablets	[8]
Sorghum	• Beverages like grain tea • Snacks like cookies • Animal feed	[9]

3. ESTABLISHMENT OF CELL SUSPENSION CULTURE

Growth of cells in starchy crop suspension cultures required different parameters according to their variation and purpose of establishment. A successful CSC depends on the optimization of growth media and growth conditions. The most common parameters that affect the growth of CSC are the type of media used and growth conditions such as inoculum size and agitation rate. Table 2 summarizes the establishment of CSC from different starchy crops such as rice (Oryza sativa L.), sweet potato and sorghum. Different starchy crops have different requirements of growth conditions and carbon sources, PGRs, inoculum size and agitation rate are significant parameters in the growth of CSC.

Carbon source is important for the in vitro cultures to provide energy and control the osmotic potential of the medium [13]. Sucrose and glucose are normally used for the CSC [13]. Fructose may be used but it can result in a slower growth of cells. This is due to the nature of plants that commonly absorb glucose faster including hydrolysis of sucrose

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compared to fructose [13]. Generally, the optimal concentration of sucrose for the growth of callus or cell suspension culture is between 20 – 40 g/L as shown in Table 2. However, for morphogenesis and plantlet development, this optimal concentration may not be suitable [14]. This is due to a large number of interaction elements in plants like mechanical stimuli, biochemical signalling or genetic prerequisites. Thus, experiments based on a statistical approach are recommended to study the optimal type and concentration of carbon sources [13]. As stated in Table 2, the most frequently supplied carbon source is sucrose with a concentration of 30 g/L.

Table 2: Establishment of cell suspension culture from different starchy crops

Starchy crops	Applications of CSC	Type of medium	Carbon source	PGRs	Inoculum size	Agitation rate	Reference
Oryza sativa L.	Genetic engineering	N6 medium	30 g/L sucrose	• 2.0 mg/L 2,4-D • 0.02 mg/L Kinetin	10%	100 rpm, dark, 28 °C	[2]
Oryza sativa L.	Genetic engineering	N6 medium	30 g/L sucrose	• 2.0 mg/L 2,4-D • 0.02 mg/L Kinetin	10%	100 rpm, dark, 28 °C	[10]
Sweet potato (*Ipomoea batatas L.*)	Molecular biology	MS medium	20 g/L sucrose	• 0.5 mg/L 2,4-D • 0.3 mg/L Kinetin	50%	120 rpm, dark, 25 °C	[11]
Sorghum bicolor L.	*In vitro* plant regeneration	MSMO	30 g/L sucrose	• 3 mg/L 2,4-D • 2.5 mg/L NAA	40%	130 rpm, orbital shaker, dark, 27 °C	[3]
Oryza sativa L.	Molecular biology	MS medium	60 g/L sucrose	• 60 mM 2,4-D • 2.5 mM 2-(N-morpholino) ethanesulfonic acid (MES).	50%	100 rpm, dark, 23 °C	[12]

PGRs are usually needed in the growth or production media since they affect the management of vegetative and reproduction growth of plants [13] [15]. Concentrations of PGRs during cell division, expansion, and differentiation have an impact on how quickly and fully plants grow and develop. The end products can also be affected by the presence of PGRs, the development stage of the target cells or tissues, the concentration of other PGRs, the target plant's health, nutrition, and water status, as well as the physical environment. The most used PGRs for the growth and development of plants are auxin and cytokinin. The auxin is used for the stimulation of root formation while cytokinin is used to stimulate the shoot formation [15]. The PGRs can either be used separately or combined for the cell's growth. Examples of auxins are indole-3-acetic acid (IAA, a natural auxin) and the synthetic auxins, 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) while cytokinins are kinetin, zeatin and benzylaminopurine. The concentration range can be between $10 - 7$ and $10 - 5$ M [5]. From Table 2, 2,4-D is the most used for different types of starchy crops and their applications in combination with another PGR.

This is due to the important roles of 2,4-D in stimulating the cell dedifferentiation process, organogenesis, and callus culture maintenance [16].

For any plant species, the initial inoculum density is crucial for generating a cell suspension culture. This is due to interactions that take place both between individual cells and the culture media, which have an impact on the cultures' biological environment [13]. To maintain active cell development after subculture, the inoculum density must stay above the minimal cell density. It is a fact that cell growth does not take place when the minimum cell density is reached or when there is a growth lag phase. The minimal cell density can be influenced by the cell line, components of the culture medium, and cell growth rate, whereas the optimal inoculum density depends on the species. Generally, a high cell density is created by a large inoculum size to establish the growing cell suspension culture. However, a low inoculum density also can initiate cell suspension culture when a conditioned medium is used. A conditioned medium is a medium that contains the cells' secreted components that are required for another set of cells [17]. The inoculum size of cells can be either 0.1 g (low) or 2.0 g (high) per 33–50 mL of medium [13]. Table 2 shows the difference in inoculum size which was between 10 – 50 %. The difference in the inoculum size may be due to the number of cells needed to be subcultured into a new media [18]. A suitable size of inoculum can prevent a "staling" that affects the growth of cells and increases nutrient depletion in media. Thus, the minimum inoculum size needed for the cell suspension culture are depending on the species and its culturing conditions.

The final parameter is agitation speed which functions to increase the multiplication rate of cell growth by increasing the gaseous exchange. This agitation is used to supply oxygen for the proliferation of cells as well as promote homogeneity in terms of nutrient supply and cell biomass due to cell dispersion [13]. High shaker speeds may be detrimental to the viability of plant cells because they may be susceptible to shear [15]. The size of cell aggregates produced as well as the amount of oxygen supplied can be affected by the agitation frequency. The most optimal shaker speed used for tissues in suspension culture is around 30 – 150 rpm while for CSC, the optimal agitation speed required is around 90 – 125 rpm [13]. From Table 2, the most used agitation speed is 100 rpm under room temperature with different purposes of study. This can be due to the physical characteristics of the studied cells like the size, thick cellulose–based cell wall or the presence of a large vacuole leading to the hydrodynamic stress on the cells caused by the agitation of cells [19].

4. CONCLUSION

In conclusion, culture parameters play an important role in starchy crops' cell suspension culture based on their application and purpose. Different plants require different establishment cell suspension culture protocols that help in the growth of cells and achieving the objectives of establishment. Understanding the culture parameter's role can help in generating an optimized condition for initiation of cell suspension culture.

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