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Voluminous Calli

About Addressing Amenable Assets by Means of a Plant Cell Culture Protocol

Plant cell culture technology enables the growing and cultivating of cells *in-vitro* outside of a living organism and its natural environment. The technology is based on standard protocols that serve as an intelligible infrastructure. The protocols are able to accommodate any plant cell with very few requirements. Friedrich Constabel and Jerry Shyluk name an overview of required materials and facilities for the initiation, nutrition and maintenance of a cell culture.

> *"A fully equipped tissue culture laboratory should contain instrumentation for media preparation plus apparatus for distilled water, autoclave, dishwasher, and a laminar air flow cabinet for tissue transfer. Growth rooms should allow for a predetermined light regime, temperature and humidity control and an alarm system."*[1]

Sterility of the bioreactor and the equipment are primarily important in order to avoid contamination of the cell cultures.

> *"Glassware has been replaced by plastic ware to the extent that only the latter deserves a description. Plastic labware is safe, dependable, can be presterilized and disposable or reusable, may be autoclavable, and is virtually indestructible."*[2]

The sterile infrastructure enables a local channel. It establishes an enclosure—a fence, a filter—to exclude the noise from the surrounding environment. Thereby the protocol aims not to control an objective environment. It does not hard-wire a relationship with nature that is seen as pre-given or pure. The protocol rather opens an abstract space for logistical movements upon a generic nature. It allows us to get in touch with plant cells in a mediate, a cultivated manner.

> *"There are many aspects of the culture environment that can influence growth and organized development. These include (a) the physical form of the medium, (b) pH, (c) humidity, (d) light, (e) temperature, and (f) the gaseous atmosphere."*[3]

Fig. 1 Callus induction

Fig. 2 Callus formation

The protocol starts with the preparation of the nutrient media.
The media is the key factor for the development of every cell culture.

> *"Success in plant cell culture is largely determined by the quality of nutrient media. No other factor has received as much attention and, as a result, numerous formulations have been published leaving confusion for any beginner. A systematic approach of nutritional requirements of tissues cultured in vitro in the 60's has led to acceptance of the fact that employment of one to three media formulations will permit to at least initiate a culture of plant tissue in vitro. Optimization of growth and plant regeneration from cultured cells and tissues may require modifications rather than novel formulations of nutrient media. Formulations designed by Murashige and Skoog (1962) and revised by Linsmaier and Skoog (1965), Gamborg et al. (1968), and by Schenk and Hildebrandt (1972) can be regarded as standard."*[4]

Standardised medias build the basis and are modified due to the specific requirements of each cell line by adding substances like vitamins and phytohormones.

> *"According to a formula chosen, chemicals are dissolved in water of about half the final volume of the medium. Once all ingredients have been added, the medium is brought to near volume and the pH is adjusted. Finally, the medium is brought to its precise volume."*[5]

Phytohormones are responsible for the regulation of growth of the cell culture.

> *"Auxins and cytokinins are the two types of phytohormones most often needed in culture. The concentration and ratio of cytokinins and auxins in the medium often control the type and amount of growth which occurs in culture."*[6]

This encoding of the medium prepares a stage. Thereupon the protocol establishes a callus culture, a noisy mass of dedifferentiated totipotent cells.

> *"Agar (0.6–1.0%), agarose (0.6–0.8%), or gelrite (0.1–0.3%) are added to nutrient media and heated to boiling once before dispensing in jars and autoclaving, or before autoclaving and subsequent dispensing in petri dishes."*[7]

The protocol proceeds with the selection of a specific part of the plant for the transfer to the nutrient media.

> *"The process of dissection and culture of small organs or tissue sections is referred to as explantation."*[8]

This choice marks an origin and adjusts the vector for the course of the unfolding of the cell culture.

> *"Origin and size of explanted tissue determine the development of a culture."*[9]

The next step includes the sterilisation of the plant material.

> *"Explanted cells, tissues, and organs as well as their environment must be sterile."*[10]

The carefully scribed explants are positioned on the media in Petri dishes.

> *"The majority of explants are maintained on solid media, solidification being achieved by media supplements of 0.6–0.8% agar."*[11]

The explants plated on the media develop callus cells at the position where the surface was scribed.

> *"Within 2–3 weeks of culture, explants show new growth across the surface of the explant depending on the distribution and mitotic activity of the parenchyma residing in the excised tissue."*[12]

In order to maintain the continuity of callus growth, the sub-cultivation with the transfer of callus cells onto fresh media is necessary. Thereby the dry weight of the cell biomass enables the determination of the growth rate of the cells.

> *"When cultured for several weeks, any callus will show signs of aging, noted as deceleration of growth, necrosis or browning, and finally desiccation. Transfer of healthy, vigorous callus pieces about 5 mm in diameter to 30 ml fresh medium (subculture) in 120-ml jars at intervals of 4–6 weeks will maintain the callus."*[13]

The growth rate augurs well for a temporary stability within the channel of the cell culture. It allows for a clear measure. A quantification of life. It describes the doubling time of the cells and serves as a key index alongside which we can learn about the condition of the cell culture. This, however, merely means the channel is on—a measure like the volume in music. A mass, a bulk of blank sheets. Though so far this says nothing about the quality, the content, the meanings of the messages. With the calli, the protocol starts with noise, the generic, with chance. It addresses plant cells as a generic ground from which we can cast off, breed and contract novel sensible natures. This necessitates further mediations—a communication with a potentially amenable asset.

1 Friedrich Constabel and Jerry P. Shyluk, 'Initiation, Nutrition, and Maintenance of Plant Cell and Tissue Cultures', in *Plant Cell and Tissue Culture*, Eds. Indra K. Vasil and Trevor A. Thorpe (Dordrecht: Springer Netherlands, 1994), 4, https://doi.org/10.1007/978-94-017-2681-8.
2 Constabel and Shyluk, 5.
3 Trevor A. Thorpe, 'Morphogenesis and Regeneration', in *Plant Cell and Tissue Culture*, Eds. Indra K. Vasil and Trevor A. Thorpe (Dordrecht: Springer Netherlands, 1994), 22, https://doi.org/10.1007/978-94-017-2681-8.
4 Constabel and Shyluk, 'Initiation, Nutrition, and Maintenance of Plant Cell and Tissue Cultures', 6.
5 Constabel and Shyluk, 7.
6 Thorpe, 'Morphogenesis and Regeneration', 21–22.
7 Constabel and Shyluk, 'Initiation, Nutrition, and Maintenance of Plant Cell and Tissue Cultures', 9.
8 Constabel and Shyluk, 10.
9 Constabel and Shyluk, 10.
10 Constabel and Shyluk, 10.
11 Constabel and Shyluk, 11.
12 Constabel and Shyluk, 12.
13 Constabel and Shyluk, 12.

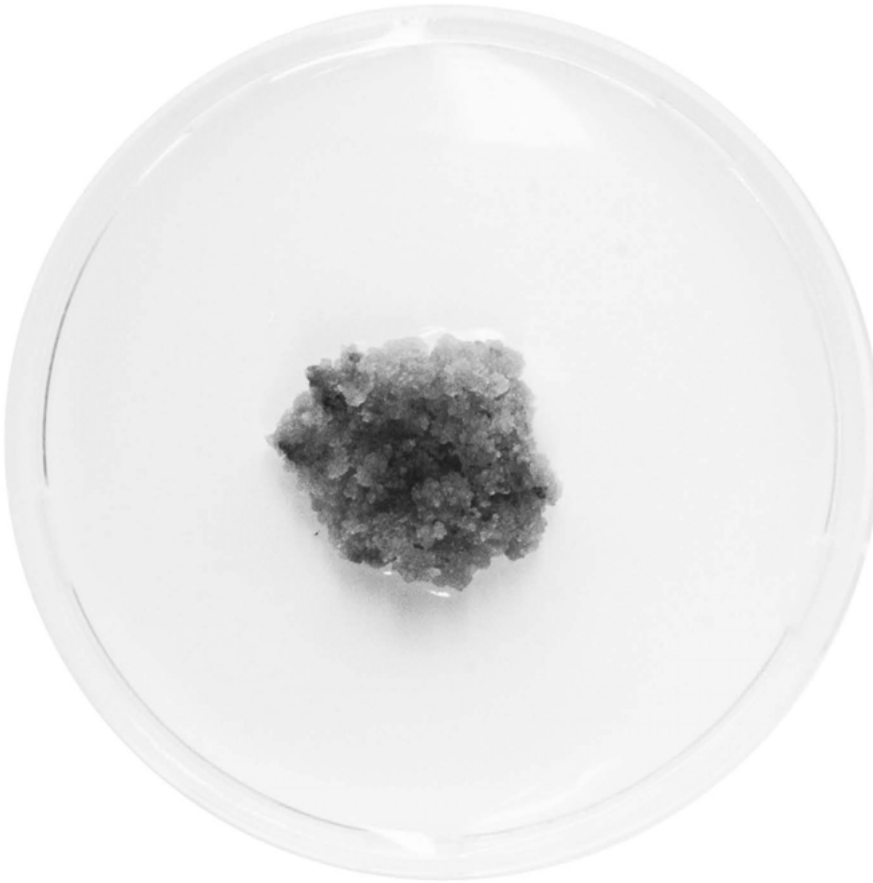

Fig. 3 Callus