PLANT CELL CULTURE

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Summary

Plant cell culture is the basis of many different technologies that are now proving to be of great benefit to many disciplines. The ability to culture plant cells or tissues is essential to the success of all of these techniques. This paper introduces the basics of plant cell culture, and discusses the techniques, which utilize the ability of plant cells to be cultured. All of these techniques have an agricultural application and are being used throughout the world to improve agricultural productivity. Many of the techniques discussed here are crucial for genetic transformation research. These techniques range from the ability to produce plant cells in a form in which they can be transformed, to the regeneration of those transformed cells. The effective and efficient use of such techniques all require a basic understanding of plant cell culture.

1. Introduction

The origin of plant cell culture derived from an interest in determining how cells would behave when isolated from the whole plant. In the wild, certain plants are capable of

regeneration from small pieces of severed tissue, for example, dandelions proliferate from isolated roots, and *Begonia* plantlets directly from leaf tissue. These observations aroused an interest in the plasticity of plant development, and the potential for cell development, if removed from the 'control' of the whole plant.

In 1902, Gottlieb Haberlandt spoke of his vision for cell biology in the future:

To my knowledge, no systematically organized attempts to culture isolated vegetative cells from higher plants in simple nutrient solutions have been made. Yet the results of such culture experiments should give some interesting insight into the properties and potentialities which the cell, as an elementary organism, possesses. Moreover it would provide information about the inter-relationships and complementary influences to which cells within the multicellular whole organism are exposed.

Haberlandt was never able to induce cell division in vitro, but in 1934, White cultured tomato roots on a basic medium of inorganic salts, sucrose and yeast extract. In the same year, Gautheret found that cambial tissue of Salix capraea and Populus alba could proliferate for several months once aseptically isolated, but growth was limited. In 1939, as a result of recognizing the importance of B vitamins and the auxin, indole-3-acetic acid, Gautheret reported on the unlimited growth of a cell culture of carrot, which resulted in the production of viable callus.

From this early work developed the concept of plant cell culture, enabling scientists through the manipulation of plant cells to develop techniques that would be of great benefit to a range of industries, such as agriculture and the pharmaceutical industry. Plant cell culture is a generic description used to describe the growth of microbe-free plant material in an aseptic (sterile) environment, such as sterilized nutrient medium in a test tube.

From this early work in the 20th century plant cell culture has developed far beyond what was first thought possible, and with the introduction of genetically modified organisms agriculture [see also - *Nitrogen fixation biotechnology*] we are seeing the ultimate in manipulation of plant cell culture. During the development of this area, numerous techniques have been established which have, in many cases, resulted in practical application. These techniques can be categorized according to whether they can be used for propagation, improvement, conservation and utilization of plant germplasm [see also - *Genetic engineering of plant cells*; - *Traditional Plant Breeding for Yield Improvement and Pest Resistance*].

2. The Basics of Plant Cell Culture

In plant cell culture, plant tissues and organs are grown *in vitro* on artificial media, which supply the nutrients necessary for growth. The precise composition of the culture medium will depend on what is required. Cell proliferation has different requirements to cell differentiation. However, basic growth is supported by a basic medium, which is generally composed of water, macro- and micro-nutrients, and a carbohydrate source, usually sucrose, to replace the carbon, which the plant normally fixes from the atmosphere by photosynthesis. To improve growth, media can include trace amounts of

certain organic compounds, such as vitamins, amino acids and plant growth regulators. Culture media can also contain what are known as undefined components. These include fruit juices, plant extracts, yeast extract etc. One of the most well-known of these undefined components is coconut milk, which has been a popular addition for orchid culture. Although, good results can be achieved using these ingredients, their use is not encouraged as their composition is not consistent, and can vary each time they are used. Depending on whether or not the culture medium is to be solid or liquid, a gelling agent is added.

95 percent of a culture medium is water, and therefore the quality of the water is important. It is recommended that the water is always distilled and in some cases, double-distilled. Macro- and micro-nutrients are essential for growth, and so are found in all culture media.

When the nutrient requirements of a plant are unknown, the nutrient composition as defined by Murashige and Skoog, can be used, providing that the plant is not sensitive to salt. Sugar is a very important component in any culture medium. A concentration of 1-5 percent saccharose (a disaccharide) is usually used, as this sugar is synthesized and transported naturally by the plant. The gelling agent usually used in culture media is agar. Agar, a seaweed derivative, is a polysaccharide with a high molecular mass. For medium of the optimum solidity, agar is usually added at a concentration between 0.6-0.8 percent.

Growth regulators are added according to what is required from the culture, but also depends on the type of explant and the plant species. For example, eggplants which themselves produce sufficient auxin do not need extra auxin for cell extension and/or division. Similarly, with explants producing enough cytokinin, cytokinin will not have to be added to the medium.

There are general rules that apply with the use of growth regulators but the individuality of the explant will always have some influence. Generally auxins cause cell elongation and expansion, cell division and the formation of adventitious roots, inhibition of adventitious and axillary shoot formation, and embryogenesis in suspension cultures. With low auxin concentration, adventitious root formation occurs whereas if the auxin concentration is high, callus formation is a possibility.

Cytokinins stimulate growth and development. In high concentrations they can be used to induce adventitious shoot formation, but root formation is generally inhibited. Care should be taken in the use of growth regulators as there is evidence in the literature that excessive use of certain growth regulators can lead to a large number of mutations.

Once the medium has been prepared it has to be at the correct pH. The pH used is between 5.5 to 6.0. If the pH is too high, this can stop growth and development. With a pH that is too low, gelling can be affected, as well as the uptake of some of the components. Finally the medium has to be sterilized, and this usually takes place in an autoclave. Providing exposure is sufficient, pressurized steam can destroy all micro-organisms. The conditions for sterilization are 20 mins at 121°C and 15psi.

Figure 1. Sterile room, Regional Germplasm Centre, Secretariat of the Pacific Community, Fiji.

The rate at which cultures grow can be influenced by the physical nature of the medium. Liquid medium is often chosen because it can result in faster rates of growth [see also - *Microbial Cell Culture*]. This is because a greater surface area of the explant is in contact with a liquid, and, providing the medium is agitated, the diffusion gradients between it and the explant are reduced. With some cultures, transfer to liquid medium after an initial stage of establishment on a solid medium works well. Bioreactors can be used with liquid medium when a mass propagation system is required. Some success has been achieved in the use of these, but there can be problems with hyperhydricity.

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Biographical Sketch

Mary Taylor obtained her Masters in Horticulture and PhD in Plant Tissue Culture from the University of Bath in UK. The past 8 years she spent as the Regional Tissue Culture Adviser with an EU-funded regional agricultural programme [Pacific Regional Agricultural Programme - PRAP]. Most of these years she was based in Western Samoa at the University of the South Pacific Campus, but worked also with other countries in the region, such as Fiji, Tonga, Vanuatu, Soloon Islands, Papua New Guinea, Tuvalu and Kiribati. Her present position is as Tissue Culture Specialist for an AusAID funded Taro Genetic Resources Project which again has a regional impact.

The TaroGen office is based at the Secretariat of the Pacific Community (SPC) in Suva, Fiji. In the early stages of her project, a Regional Germplasm Centre [RGC] was established at SPC, where she also acted as an adviser. The RGC is being developed to house collections of all the important crops in the Pacific, in vitro storage (slow growth and cryopreservation), field conservation, in situ conservation, intellectual property rights issues, and plant genetic resources issues.