Plant Tissue Culture

Historical Developments and Applied Aspects

H R Dagla

The success of plant biotechnology relies on the fundamental techniques of plant tissue culture. Understanding basic biology of plants is a prerequisite for proper utilization of the plant system or parts thereof. Plant tissue culture helps in providing a basic understanding of physical and chemical requirements of cell, tissue, organ culture, their growth and development. Establishment of cell, tissue and organ culture and regeneration of plantlets under *in vitro* conditions has opened up new avenues in the area of plant biotechnology.

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1. Introduction

Plant tissue culture is a technique of culturing plant cells, tissues and organs on synthetic media under aseptic environment and controlled conditions of light, temperature, and humidity. The development of plant tissue culture as a fundamental science was closely linked with the discovery and characterization of plant hormones, and has facilitated our understanding of plant growth and development. Furthermore, the ability to grow plant cells and tissues in culture and to control their development forms the basis of many practical applications in agriculture, horticulture industrial chemistry and is a prerequisite for plant genetic engineering [1].

2. History

History of plant tissue culture is a record of systematic efforts by botanists to culture excised plant tissues and organs to understand their growth and development under controlled conditions.

2.1 *Cell Culture*

The idea of experimenting with the tissues and organs of plants in

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Box 1. Plant Tissue Culture Media

Success in plant cell culture is largely determined by the quality of nutrient media. A medium containing 'chemically-defined' compounds is referred to as a 'synthetic medium'. One of the earliest plant tissue culture media is the root culture medium of White (1939). The formulation prepared by Murashige and Skoog (1962) (MS Medium) and revised by Linsmaier and Skoog (1965), Gamborg *et al*. (1968) and Schenk and Hildbrandt (1972) can be regarded as a standard medium. MS medium was designed to test the effect of organic supplements on tissue cultures. The medium therefore was standardized with regard to inorganic nutrients and formulated for tobacco pith tissue. B_5 medium (Gamborg *et al*.) formulated for growing soyabean tissues and SH medium for growth of friable callus serve as feedstock for cell suspension culture and production of protoplasts [23].

isolation under controlled laboratory conditions arose during the later part of the nineteenth century. German botanist Gottlieb Haberlandt was the first person to culture isolated, fully differentiated cells in 1898 [2]. He selected single isolated cells from leaves and grew them on Knop's (1865) salt solution with sucrose. Haberlandt succeeded in maintaining isolated leaf cells alive for extended periods but the cells failed to divide because the simple nutrient media lacked the necessary plant hormones. Although he could not demonstrate the ability of mature cells to divide, he was certain that in the intact plant body, the growth of a cell simply stops due to a stimulus released by the organism itself, after acquiring the features required to meet the need of the whole organism. Haberlandt's vision was to achieve continued cell division in explanted tissues on nutrient media (*Box* 1); that is, to establish true, potentially perpetual tissue culture. This goal was attained only after the discovery of auxins [3].

Although Haberlandt was unsuccessful in his attempts to culture cells, he foresaw that they could provide an elegant means of studying morphogenesis. And the result of such culture experiments should give some interesting insight into the properties and potentialities which the cell as an elementary unit of life possesses.

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2.2 *Organ Culture*

In the early part of the 20th century, efforts in growing excised

plant tissues in culture continued with the development of sterile working methods (*Box* 2) and discovery of the need for B vitamins and auxins for tissue growth. In 1922, Robbins (USA) and Kotte (Germany) reported some success with growing isolated root tips. The first successful experiment to maintain growth and cell division in plant cell culture was conducted by White (1934) who established cultures of isolated tomato roots under aseptic conditions. White's medium was simple, containing only sucrose, mineral salts and yeast extract, which supplied vitamins. The cultured roots maintained their morphological identity as roots with the same basic anatomy and physiology. This happened only because excised plant organs on nutrient media are capable of synthesizing hormones necessary to maintain cell division. Ball (1946) obtained whole plants from cultured shoot meristem. This heralded the present day method of *in vitro* vegetative multiplication. Ball is considered the father of so-called micropropagation [4]. Morel and Martin [5] cultured shoot meristem of virus-infected plants to raise healthy plants from *Dahlia*. The cells of the shoot tip of virus infected plants are free of virus or contain a negligible number of virus. Axillary bud proliferation has immense practical applications for large-scale clonal propagation of plants of importance in agriculture, horticulture and forestry.

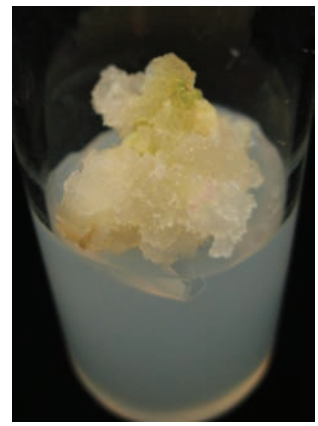
Box 2. Sterilization and Aseptic Inoculation

Culture vial containing the medium is closed with a cotton plug and then autoclaved (steam heating under pressure) at 1.06 kg cm^{-2} (121°C) for 15–40 min. Explants (excised plant parts) are surface sterilized, using sterilizing agents such as sodium hypochlorite or mercuric chloride solutions, before they are planted on the nutrient medium under aseptic conditions in a laminar airflow cabinet.

2.3 *Tissue or Callus Culture*

A mass of unorganized protoplasmic (undifferentiated living) cells is known as 'callus' (*Figure*1a). White (1939) cultured tissue of plant tumors (galls) that were produced by a hybrid between *Nicotiana glauca* and *N. langsdorffii* on the same medium that was used for tomato roots. Proliferated cell masses from the original explants were divided and subcultured. Gautheret and Nobecourt in 1939 reported unlimited growth of cultures derived from carrot tap root tissue, using indole-3-acetic acid (IAA). The goal at that time was to establish unlimited growth of a culture by repeated subcultures. Much effort was devoted to determine the nutritional requirements for sustained growth. White and Braun (1942) initiated studies on crown gall and

Figure 1a. Callus multiplication.



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tumour formation in plants and Skoog (1944) initiated work on organogenesis in tobacco callus. Although continuously growing cultures could be established in 1939, the objective of Haberlandt to induce cell division in isolated vegetative cells, was not achieved, because the tissues used by them were not meristematic in nature.

The most significant event that led to advancement in the field was the discovery of the nutritional properties of coconut milk. Van Overbeek and his coworkers (1941) cultured isolated embryo of *Datura* on a medium containing coconut milk. The combination of 2,4-D (2, 4-dichlorophenoxy acetic acid) and coconut milk had a remarkable effect on stimulating growth of cultured carrot and potato tissues [6–8]. In a search for cell division factor, Skoog's group located such a factor in degraded DNA preparation. It was isolated, identified as 6-furfurylaminopurine, and named it Kinetin [9]. The related analogue, 6-benzylaminopurine, was then synthesized and that too stimulated cell division in cultured tissues. The generic term 'cytokinin' was given to this group of 6-substituted aminopurine compounds that stimulate cell division in cultured plant tissues. Later Zeatin was discovered as a natural plant hormone. Skoog and Miller [10] advanced the hypothesis that shoot and root initiation in cultured callus can be regulated by specific ratios of auxin and cytokinin. The availability of cytokinins made it possible to induce divisions in cells of mature and differentiated tissues.

At this stage, the dream of Haberlandt was realized partially, for he foresaw the possibility of cultivating isolated single cells. Only small pieces of tissue could be grown in cultures. Further progress was made by Muir [11] who transferred callus of *Tagetes erecta* and *Nicotiana tabacum* to liquid medium (culture medium devoid of agar-agar) and agitated the cultures on a shaking machine, to break the tissue into single cells and small cell aggregates. Muir *et al*. (1954) succeeded in making single cells to divide by placing them individually on separate filter papers resting on top of a well-established callus culture. Callus tissue separated from the cells by thin filter paper, supplied the neces-

sary factor(s) for cell division. Jones (1960) *et al*. designed a micro-chamber for growing single cells in hanging drops in a conditioned medium (medium in which callus has been grown previously). Using a micro-chamber and replacing the conditioned medium with a fresh medium containing coconut milk, Vasil and Hildbrandt (1965) raised whole plants starting from single cells of tobacco. They transferred single tobacco hybrid cells to a drop of culture medium on a slide, and observed separately under phase contrast microscope and photographed their observations. Cells were observed to divide and form callus which differentiated into roots and leafy shoots. However, they did not prove that the whole plants were the direct product of a single cell, rather than the product of a tissue mass within which somaclonal or other genetic changes might have taken place during growth.

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Finally, Haberlandt's prediction, that one could successfully cultivate artificial embryos from vegetative cells, was proved by the research of Backs-Husemann and Reinert in Berlin. They mounted isolated single cells on microscope slides and photographed repeatedly. Isolated cells divided to form a mass of embryogenic and parenchyma cells which developed into heart-shaped and torpedo-shaped embryos with recognizable cotyledons, hypocotyls and radicles [12]. Tuleke (1953) cultured pollen grains of *Ginkgo biloba* in a medium containing vitamins and amino acids and obtained cell clumps, some of which looked similar to embryos. Yamada *et al*. [13] reported that culture of *Tradescantia reflexa* anther produced haploid tissues. Guha and Maheshwari [14] reported that immature pollen grains produced embryos. Colchicine treatment can transform them into diploid fertile plants. Klercker (1892) and Kuster (1909) reported isolation and fusion of protoplasts, respectively. Cocking [15] developed enzymatic method of protoplast isolation. The method involved the enzymatic digestion of cell wall by cellulase and pectinase enzymes extracted from the fungus *Myrothecium verrucaria*. Cultured protoplasts regenerated new walls, developed colonies and eventually plantlets [16]. Protoplasts are now

used for creation of somatic hybrids within and between species and genera. The first hybrid between *N. Glauca* and *N. langsdorffii* was produced by Carlson [17]. In 1978, Melchers *et al*. [18] produced a hybrid between potato and tomato, but the hybrid was sterile. Novel application of protoplast fusion is called cybrid production, where cytoplasm of two species or genera is fused with nuclear genome of only one cell (nuclear–cytoplasmic combination).

3. Applied Aspects of Plant Tissue Culture

Establishment of plant tissue culture techniques has enabled botanists to introduce this method in major areas of plant sciences such as plant breeding, industrial production of natural plant products, conservation of germplasm and genetic engineering.

3.1 *Plant Breeding*

Establishment of cellular totipotency[1], callus differentiation and vegetative multiplication under *in vitro* conditions has opened up new dimensions in the applied field of plant sciences. Rapid vegetative propagation or micropropagation of plants of elite characteristics is possible through axillary shoot induction (*Figure* 1b) and rooting them (*Figure* 1c) *in vitro* to raise complete plantlets. Somatic embryogenesis[2] and organogenesis (callus differentiation) are other methods of micropropagation. Seedlings (*Figure* 1d) derived from mature seeds can also be used as

[1] Schwann (1839) expressed the view that each living cell of a multicellular organism would be capable of developing independently if provided with proper external conditions. Totipotency is the ability of a cell to divide and regenerate into a whole organism.

[2] Somatic embryos and synthetic seeds: Embryo produced *in vitro* using vegetative (somatic) cells is known as a somatic embryogenesis. Encapsulated (Ca-alginate covering) somatic embryo is termed as synthetic seed.

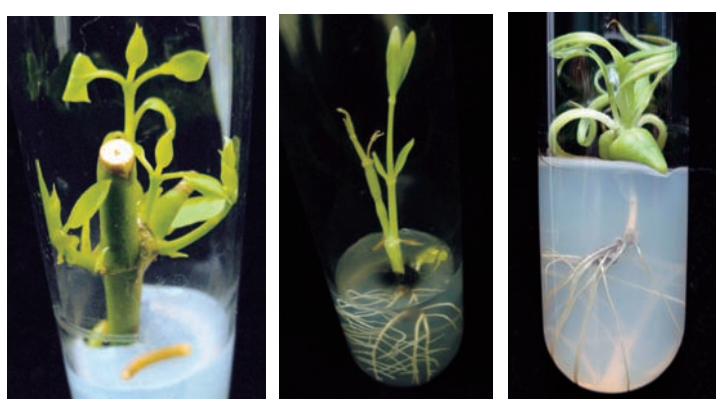

Figure 1b (left). Multiple shoots induction from nodal shoots segment.
Figure 1c (center). *In vitro* rooting of shoot to raise complete plantlet.
Figure 1d(right). *In vitro* seed germination and seedling development.

a source for large-scale multiplication of rare and endangered plant species. Virus-free plants can be raised using apical meristems of virus-infected plants. Homozygous plants can be obtained in a single generation by diploidization[3] of the haploid cells such as pollen grains. Protoplast technology has made it possible to develop somatic hybrids and cybrids of distantly related plant species and genus. Protoplasts are also a suitable material for genetic engineering of plants in a manner similar to gene transfer into bacteria. Cell culture may be an important source of induction and selection of cell variants for production of new varieties of economically important plants.

[3] Doubling the chromosomes of haploids using colchicines.

3.2 *Industrial Production of Natural Plant Products*

Plants produce a variety of natural compounds that are used as agricultural chemicals, pharmaceuticals and food additives. Cell culture technique is being used as an efficient system for production of high-value natural plant products at industrial level. In the 1950s and 1960s, great efforts were made by the Pfizer Company to culture plant cells in liquid medium (suspension culture[4]) similar to culture of microbes for production of natural plant products as an alternative to whole plants. Different kinds of bioreactors[5] have been designed for large-scale cultivation of plant cells. Culture of hairy roots produced by transformation with *Agrobacterium rhizogenes* has been shown to be a more efficient system than cell culture for the production of compounds which are normally synthesized in roots of intact plants. The first tissue culture product to be commercialized by Mitsui Petrochemical Co. of Japan is shikonin, a natural colouring substance, from the cell cultures of *Lithospermum erythrorhizon* [19].

[4] Fragmented callus transferred into a liquid medium and agitated on a shaker to provide uniform aeration and nutrients to the suspended cells.

[5] A vessel used for large scale aseptic culture of cells, embryos or shoots under controlled growth conditions.

3.3 *Conservation of Germplasm*

Successful regenerating of whole plants from somatic and gametic cells and small shoot apices, and storage of germplasm is possible by using *in vitro* techniques. This is an efficient method by which small portions of plant parts in the form of cell, tissue and organ can be stored for longer periods in a limited space, free from contamination and infection.

3.4 *Genetic Engineering of Plants*

Protocol for cell culture and regeneration of plants from single cell is a basic requirement for development of genetically modified plants. Single cell culture and regeneration of plants from single cell is possible through plant tissue culture techniques. *Agrobacterium tumefaciens*-based vectors play an important role in genetic modification of plants. Smith and Townsend [20] had shown that *A. tumefaciens*, the Gram negative soil bacteria cause crown gall disease in some plants. They observed that crown gall tissue displayed the tumourigenic character of autonomous growth on salt–sugar medium, even in the absence of growth regulators. Braun [21] suggested that the bacterium introduces a tumour-inducing property in plant genome. This was identified as Ti-plasmid [22]. Phytohormone biosynthesizing genes from T-DNA[6] of Ti-plasmid were removed to eliminate the aberrant cell proliferation ability of Ti-plasmid. *A. tumefaciens* can be used to transfer genes conferring desired traits into plant cells. This transformation system is species-specific and does not work in most monocotyledons including major cereals. Therefore, free DNA delivery techniques, such as electroporation, particle gun and microinjection are mostly used for genetic modification of cells, tissues and organs of monocots.

[6] The portion of the Ti-plasmid transferred to the plant DNA is T-DNA. It is a common practice to use *A. tumefaciens* for gene transfer.

Suggested Reading

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