An Introduction to Plant Tissue Culture: Advances and Perspectives

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Chapter 1

An Introduction to Plant Tissue Culture: Advances and Perspectives

Victor M. Loyola-Vargas and Neftalí Ochoa-Alejo

Abstract

Plant tissue culture techniques are the most frequently used biotechnological tools for basic and applied purposes ranging from investigation on plant developmental processes, functional gene studies, commercial plant micropropagation, generation of transgenic plants with specific industrial and agronomical traits, plant breeding and crop improvement, virus elimination from infected materials to render high-quality healthy plant material, preservation and conservation of germplasm of vegetative propagated plant crops, and rescue of threatened or endangered plant species. Additionally, plant cell and organ cultures are of interest for the production of secondary metabolites of industrial and pharmaceutical interest. New technologies, such as the genome editing ones combined with tissue culture and *Agrobacterium tumefaciens* infection, are currently promising alternatives for the highly specific genetic manipulation of interesting agronomical or industrial traits in crop plants. Application of omics (genomics, transcriptomics, and proteomics) to plant tissue culture will certainly help to unravel complex developmental processes such as organogenesis and somatic embryogenesis, which will probably enable to improve the efficiency of regeneration protocols for recalcitrant species. Additionally, metabolomics applied to tissue culture will facilitate the extraction and characterization of complex mixtures of natural plant products of industrial interest. General and specific aspects and applications of plant tissue culture and the advances and perspectives are described in this edition.

Key words Aseptic culture, Genetic modified organisms, Large-scale propagation, Metabolic engineering, Plant cell culture, Proteomics, Transcriptomics

1 Introduction

Plant tissue culture is a broad term that refers to the culture of any part of a plant (cells, tissues, or organs) in artificial media, in aseptic conditions, and under controlled environments. This set of techniques emerged as an experimental approach to demonstrate the cell theory, which establishes that all living organisms are constituted of cells, the basic units of structure and reproduction, and also the totipotency concept, which is defined as the genetic potential of a cell to generate an entire multicellular organism [1]. Different attempts were conducted by several researchers to investigate the

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conditions to initially achieve the growth of organs [2] or tissues [3] in an artificial nutrient culture medium [4] rather than isolated cells because of the complex nutritional and hormonal requirements they need. Nutrient solutions alone or supplemented with natural extracts were used as starting culture media, and some important results were reported [5]; however, the discovery of plant growth regulators was determinant for the successful establishment of in vitro plant tissue cultures [6, 7]. A key advance in plant tissue culture was the control of morphogenesis by using different levels and combinations of growth regulators [8], because this allowed the regeneration of entire plants, opening the possibility of using in vitro systems to study fundamental aspects of cell differentiation and development, and also for the application of tissue culture for different purposes. Some other relevant advances in plant tissue culture were the culture of meristems as a tool for getting virus-free plants [9]; the demonstration of totipotency in haploid or gametophyte cells, which made possible the faster generation of isogenic lines important for plant breeding programs [10, 11]; the rescue of hybrid embryos to overcome sexual incompatibility between plant species [12]; the enzymatic degradation of cell walls of plant cells to produce protoplasts and the fusion of these naked cells to eliminate sexual barriers between different plant species to render intraspecific or interspecific somatic hybrids [13, 14]; and the production of secondary compounds using cell or organ cultures [15], and perhaps the most relevant advance in plant tissue culture was the development and establishment of genetic transformation systems by *Agrobacterium tumefaciens* infection and through particle bombardment to allow the genetic manipulation of plant species [16] (Fig. 1).

2 Basic Principles of Cell, Tissue, and Organ Culture

Anyone who wishes to start plant tissue cultures should have in mind the following basic principles: (1) select an appropriate explant from a healthy and vigorous plant, (2) eliminate microbial contamination from the surface of the explant, (3) inoculate the explant in an adequate culture medium, and (4) provide the explant in culture with the proper controlled environmental conditions. In the case of in vitro regenerated plants, they are subjected to an adaptation process (acclimatization) in the greenhouse before the transference to ex vitro conditions.

Depending on the part of the plant that is cultured, we can refer them as cell culture (gametic cells, cell suspension, and protoplast culture), tissue culture (callus and differentiated tissues), and organ culture (any organ such as zygotic embryos, roots, shoots, and anthers, among others). Each type of culture is used for different basic and biotechnological applications.

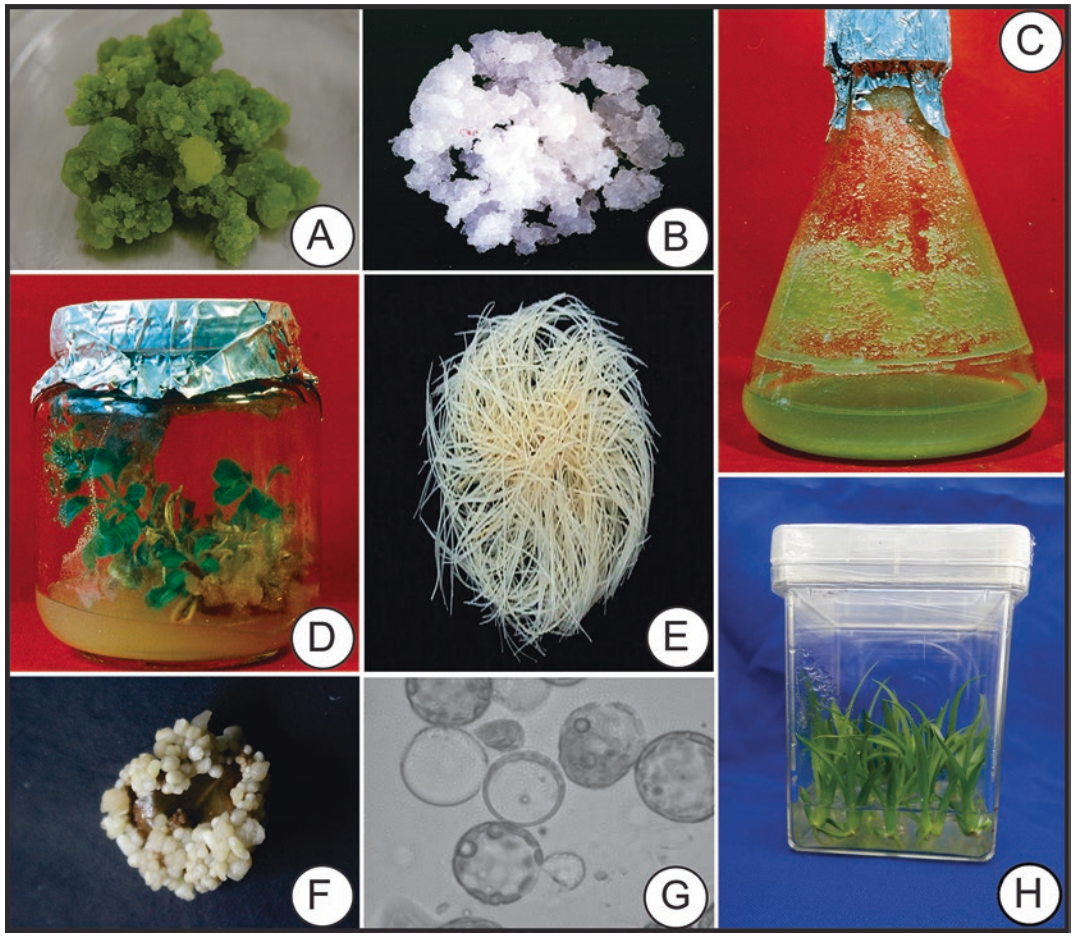

Fig. 1 (**a**) Mixotrophic callus from *Catharanthus roseus*. (**b**) Heterotrophic callus from *Catharanthus roseus*. (**c**) Suspension culture from *Catharanthus roseus*. (**d**) Regeneration of *Catharanthus roseus* plants from callus. (**e**) Root culture from *Catharanthus roseus*. (**f**) Somatic embryogenesis in *Coffea canephora*. (**g**) Protoplast from *Coffea canephora*. (**h**) Micropropagation of *Agave fourcroydes*. Pictures **a**, **b**, **c**, **d**, **e**, **f,** and **g** are from the authors' laboratories. Picture **h** is a gift from the laboratory of Dr. Clelia De la Peña, from Centro de Investigación Científica de Yucatán

3 Micropropagation

Undoubtedly, micropropagation or in vitro clonal propagation is one of the most current extended commercial applications of tissue culture (*see* Chapters 2, 8, and 10). Plant tissue culture is an excellent tool for the asexual multiplication of those species that are naturally reproduced asexually, but it is also used to overcome some problems of germination of seeds in different plant species; for example, recalcitrant species are particularly characterized for their short-seed viability (recalcitrant seeds), and therefore, asexual multiplication is a good alternative. Although tissue culture can be

applied for the micropropagation of almost any plant species, it is recommended only for those that are economically profitable. Among the plant species that are currently micropropagated at the commercial level, the ornamentals occupy the first place. Micropropagation of plants may be carried out through three different ways: (1) by promoting the proliferation of apical or axillary buds and then rooting them, (2) by inducing adventitious bud formation and its further rooting (*see* Chapters 2 and 3), and (3) by somatic embryo formation, maturation, and germination (*see* Chapters 9, 12, 13, 14, 15, and 16). Each alternative can be applied to several plant species at different efficiencies depending on the genetic background or regeneration capacity, the culture media, and the incubation conditions.

4 Plant Breeding and Genetic Improvement

Plant tissue techniques can be certainly powerful auxiliary tools for plant breeding and genetic improvement programs. Genetic variability detected in callus tissue and cell cultures can be due to genetic or epigenetic changes and represents an important possibility for recovering somaclonal variants or mutants with specific agronomic or industrial characteristics that can be exhibited at the cell or plant level [17]. Thousands or millions of cells constitute a piece of callus or a cell suspension, and they can be subjected to a selective pressure of different kinds of stresses to isolate resistant cells under controlled conditions. The recovered resistant cells may regenerate the entire resistant plants when cultured in adequate media. In this way, it is possible to generate plants resistant to drought, salinity, and cold or to biotic stress that affects crop yield [18]. A novel protocol for the estimation of somaclonal variation using molecular markers is described in Chapter 6.

Isogenic or homozygous plants are important materials for breeding programs since they are used as parental lines to generate hybrid seeds, which when they raise plants, they have high yields. However, the generation of isogenic or homozygous lines can take five to ten cycles of self-fertilization by the traditional breeding techniques. By using microspore or anther culture, the time to produce isogenic lines may be reduced dramatically, because haploid plants can be regenerated in just one cycle of culture and then they can be diploidized by a colchicine treatment to get double-haploid plants with fixed homozygous sets of chromosomes [19, 20] (*see* Chapter 21). Anther or microspore culture can be also used to fix the characteristics of hybrid plants generated by parental crosses and conventional techniques.

Embryo rescue and culture allow the recovery of hybrid plants from partially sexual compatible species. After cross-pollination between two different species, the development of the hybrid embryo occurs,

but the endosperm not necessarily accompanies the whole process of seed development, and at certain step, the hybrid embryo aborts; it is in that moment that the embryo can be rescued and cultured for further development [21, 22] (*see* Chapter 20).

Intra- or interspecific hybrid plants can be also generated in sexual incompatible plant species through somatic hybridization using protoplasts from two different sources, which are fused by physicochemical methods. The hybrid cells are cultured to regenerate hybrid plants. Different somatic hybrid plants have been generated and described in the literature [23–27].

5 Genetic Engineering

Plant genetic engineering is possible thanks to the use of plant tissue culture systems combined with recombinant molecular biology techniques. The goal of plant genetic engineering is to manipulate genetic material from different organisms in such a way to have specific sequences coding for specific genes that confer particular characteristics when they are introduced and integrated into a plant genome. Once a gene of interest is isolated, a construct is prepared in an appropriate vector to carry out the genetic transformation using either biological (*Agrobacterium tumefaciens*-mediated infection) (*see* Chapter 33) or physical methods (usually microparticle bombardment). Genetic transformation has been achieved with important crops such as corn, wheat, cotton, rice and soybean, among others, and millions of hectares are currently planted with transgenic crops resistant to pests [28] or herbicides [29]. A reduction in the applications of toxic insecticides (organophosphorus insecticides) to control several pests is expected with the use of transgenic plants resistant to insects. Besides biotic factors, crop production and yield are much more frequently affected by abiotic factors (water stress, salinity, and cold, among others). Plants have evolved adaptation mechanisms to abiotic factors, but, in general, they are quite complex because they involve physiological, biochemical, and molecular processes. However, transgenic resistant plant crops to drought or salinity have been already generated [30–34], opening new opportunities of manipulation of complex abiotic resistant traits to cope with different environmental stresses. Genetic transformation has been also a powerful approach in basic science to carry out functional studies of plant genes (*see* Chapter 33).

6 Genome Editing

In the last decade, different genome editing techniques based on the use of sequence-specific nucleases have allowed precise manipulation of target genomic sequences opening the possibility of

creating specific desirable mutations [35, 36]. Genome editing technology combined with plant tissue culture and genetic transformation has started to revolutionize the breeding and improvement programs of several crops. One of these technologies involves the CRISPR/Cas9 genome editing system [37–39]. Comparatively with genetic transformation, genomic editing technologies do not imply the use of foreign DNA to make a genetic change in the receptor plant, but the genetic change is carried out in the own genome of the plant species to be genetically modified [40]. A review of this novelty technology is described in Chapter 7. Examples of successful genetically edited modified plants using CRISPR/Cas9 include important crops such as rice [41, 42], wheat [43], corn [44], tomato [45], and potato [46], among others. Genome editing systems are also currently of high value for functional gene studies [47, 48].

7 Omics and Plant Tissue Culture

Genomics (the study of gene structure, function and regulation, and related techniques), transcriptomics (the study of the transcriptome or the set of genes that are transcribed in an organism), proteomics (the study of the set of proteins translated in an organism), and metabolomics (the study of all metabolites present in an organism) have become essential for the study of biological processes in plants. The knowledge on plant genomes, transcriptomes, proteomes, and metabolomes has impacted favorably in the comprehension of complex developmental processes, such as in vitro organogenesis, embryogenesis, or dedifferentiation, and the genetic changes induced during in vitro conditions [49–51] (*see* Chapters 24, 25, and 29). Additionally, metabolomics can be very useful to investigate secondary metabolism not only during morphogenetic processes but mainly in cell, tissue, and organ cultures of plant species producing secondary metabolites of industrial and pharmaceutical interest [52, 53] (*see* Chapter 32).

8 Epigenetics in Plant Tissue Culture

Epigenetic changes (heritable changes in gene function that do not involve changes in the DNA sequence) affecting in vitro plant regeneration and also explaining the variation frequently observed in either cells or regenerated plants have been reported [54–61]. Due to the impact of these epigenetic changes on tissue cultures, it was considered convenient to include in this edition protocols regarding the analysis of histone modifications and gene regulation (*see* Chapter 26).

9 Preservation and Conservation of Plant Germplasm

Plant germplasms are the genetic resources that are collected and conserved for plant breeding and crop improvement programs, and they represent really true preserved treasures of genetic variability from which plant breeders start looking for specific desirable characteristics to be selected to increase the yield of crops. Plant germplasm of important crops such as corn and wheat are maintained as seed collections under low temperatures at the Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center; CIMMYT) in México, whereas rice germplasm is concentrated at the International Rice Research Institute (IRRI) in the Philippines. Plant germplasms of vegetatively propagated crops such as potato (*Solanum tuberosum* L.) and sweet potato (*Ipomoea batatas* L.) are preserved in the form of tubercles or under tissue culture conditions at the Centro Internacional de la Papa (International Potato Center; IPC) in Peru. Plant tissue culture offers excellent alternatives for the conservation of germplasm of those crops that are vegetatively propagated since thousands of plantlets may be conserved in small spaces under controlled conditions that can reduce the growth of cultures (minimum growth) or can even stop completely their growth (cryopreservation). Cryopreservation protocols for shoot tips of pineapple and pollen of bromeliads are described in Chapters 18 and 19.

10 Future Perspectives

Plant cell, tissue, and organ cultures have been applied to a range of different purposes including micropropagation, which is the most extended and successful application at commercial level and surely will continue in the future, and genetic engineering of important crops to confer tolerance mainly to pests and herbicides enabling the increase in production and yield with less applications of toxic insecticides and herbicides in millions of hectares worldwide. A significant impact is predicted in the production of different transgenic crops resistant or tolerant to drought, salinity, or cold under these stress conditions in the near future. Additionally, genetic transformation will be certainly a strategic tool for facing the global warming and its consequences by generating transgenic plants resistant to abiotic factors. Genetic engineering is still expected to contribute to the development of transgenic crops with increased nutritional or nutraceutical value or resistant to diseases caused by fungi, bacteria, or viruses. Plant metabolic engineering contribution to the development of more metabolically efficient crops [62, 63] or with modified biochemical pathway leading to the production of commercial secondary

metabolites has been slow and modest, but it should have great promise to regulate the biosynthesis of target diverse secondary metabolites of industrial and pharmaceutical interest [64, 65]. Much more difficult is to evaluate quantitatively the impact that tissue culture has had or will have on plant breeding and crop improvement using embryo rescue, double-haploid generation, or somatic hybridization, but of course they will be contributing to get improved hybrid crops to increase productivity. Somaclonal variation in tissue cultures has been employed to rescue or recover interesting materials that have led to the generation of new varieties [66] and undoubtedly will continue to be applied in the future for the isolation of somaclones bearing polygenic novel traits in which the mechanisms underlying complex agronomical characteristics are unknown.

Genome editing techniques have opened a new and wide avenue for the second green revolution and certainly will allow the creation of new and novel plant varieties with useful agronomic traits through the fine manipulation of specific genetic changes in important crop species [67]. The development of high-throughput genome and transcriptome sequencing techniques, the application of protein separation and sequencing, and the improvement of extraction, separation, and identification of metabolites, as well as the availability of data in public databases, have helped to decipher genome organization, gene function and regulation, and prediction of protein function and to know the set of metabolites produced in different plant species. Omics have therefore become fundamental tools for the study of basic biological processes in plants. Integration of omics is desirable for a better understanding of whole biological phenomena. It is evident that omics will be of great benefit to investigate in vitro morphogenetic processes and should facilitate the establishment of more efficient in vitro plant regeneration protocols if master control genes of differentiation and development are identified and characterized. On the other hand, the combination of different omics should enable the metabolic engineering of interesting biochemical pathways in order to manipulate specific characteristics for the optimization and production of secondary metabolites of industrial and pharmaceutical importance.

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