Cell Culture and Somatic Cell Genetics of Plants

VOLUME 2

Cell Growth, Nutrition, Cytodifferentiation, and Cryopreservation

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1985

ACADEMIC PRESS, INC.

Harcourt Brace Jovanovich, Publishers

Orlando San Diego New York Austin
London Montreal Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data
(Revised for vol. 2)
Main entry under title:

Cell culture and somatic cell genetics of plants.

Includes bibliographies and index.
Contents: v. 1. Laboratory procedures and their applications.
1. Plant cell culture–Collected works. 2. Plant cytogenetics–Collected works. I. Vasil, I. K.
QK725.C37 1984 581'.07'24 83-21538
ISBN 0–12–715002–1 (v. 2 : alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

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To my parents,
Pushpalata and the late
Lal Chand Vasil

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General Preface

Recent advances in the techniques and applications of plant cell culture and plant molecular biology have created unprecedented opportunities for the genetic manipulation of plants. The potential impact of these novel and powerful biotechnologies on the genetic improvement of crop plants has generated considerable interest, enthusiasm, and optimism in the scientific community and is in part responsible for the rapidly expanding biotechnology industry.

The anticipated role of biotechnology in agriculture is based not on the actual production of any genetically superior plants, but on elegant demonstrations in model experimental systems that new hybrids, mutants, and genetically engineered plants can be obtained by these methods, and the presumption that the same procedures can be adapted successfully for important crop plants. However, serious problems exist in the transfer of this technology to crop species.

Most of the current strategies for the application of biotechnology to crop improvement envisage the regeneration of whole plants from single, genetically altered cells. In many instances this requires that specific agriculturally important genes be identified and characterized, that they be cloned, that their regulatory and functional controls be understood, and that plants be regenerated from single cells in which such gene material has been introduced and integrated in a stable manner.

Knowledge of the structure, function, and regulation of plant genes is scarce, and basic research in this area is still limited. On the other hand, a considerable body of knowledge has accumulated in the last fifty years on the isolation and culture of plant cells and tissues. For example, it is possible to regenerate plants from tissue cultures of many plant species, including several important agricultural crops. These procedures are now widely used in large-scale rapid clonal propagation of plants. Plant cell culture techniques also allow the isolation of mutant cell lines and plants, the generation of somatic hybrids by protoplast fusion, and the regeneration of genetically engineered plants from single transformed cells.

Many national and international meetings have been the forums for discussion of the application of plant biotechnology to agriculture. Neither the basic techniques nor the biological principles of plant cell culture are generally included in these discussions or their published proceedings. Following the very enthusiastic reception accorded the two volumes entitled "Perspectives in Plant Cell and Tissue Culture" that were published as supplements to the *International Review of Cytology* in 1980, I was approached by Academic Press to consider the feasibility of publishing a treatise on plant cell culture. Because of the rapidly expanding interest in the subject both in academia and in industry, I was convinced that such a treatise was needed and would be useful. No comprehensive work of this nature is available or has been attempted previously.

The organization of the treatise is based on extensive discussions with colleagues, the advice of a distinguished editorial advisory board, and suggestions provided by anonymous reviewers to Academic Press. However, the responsibility for the final choice of subject matter included in the different volumes, and of inviting authors for various chapters, is mine. The basic premise on which this treatise is based is that knowledge of the principles of plant cell culture is critical to their potential use in biotechnology. Accordingly, descriptions and discussion of all aspects of modern plant cell culture techniques and research are included in the treatise. The first volume describes every major laboratory procedure used in plant cell culture and somatic cell genetics research, including many variations of a single procedure adapted for important crop plants. Two subsequent volumes in preparation are devoted to the nutrition and growth of plant cell cultures and to the important subject of generating and recovering variability from cell cultures. An entirely new approach is used in the treatment of this subject by including not only spontaneous variability arising during culture, but also variability created by protoplast fusion, genetic transformation, etc. Future volumes are envisioned to cover most other relevant and current areas of research in plant cell culture and its uses in biotechnology.

In addition to the very comprehensive treatment of the subject, the uniqueness of these volumes lies in the fact that all the chapters are prepared by distinguished scientists who have played a major role in the development and/or uses of specific laboratory procedures and in key fundamental as well as applied studies of plant cell and tissue culture. This allows a deep insight, as well as a broad perspective, based on personal experience. The volumes are designed as key reference works to provide extensive as well as intensive information on all aspects of plant cell and tissue culture not only to those newly entering the field but also to experienced researchers.

Indra K. Vasil

Preface to Volume 2

The primary objective of these volumes is to provide authoritative, up-to-date, extensive, and in-depth information on all aspects of cell culture and somatic cell genetics of plants. There is a deliberate bias, however, toward science and technology, although applied aspects are discussed and emphasized also where relevant. A key feature is the selection of individuals who have played a leading role in the development of our knowledge in this important area of biotechnology to author chapters in their fields of specialization, so that others may benefit from insight gained during long years of personal experience.

This volume forms a natural bridge between Volume 1, devoted to laboratory procedures and their applications, and Volume 3, devoted to regeneration and genetic variability. Volume 2 thus begins with a detailed historical account, going back more than two centuries, by Professor R. J. Gautheret, and includes many previously unpublished photographs of scientists who made important contributions to the field. Professor Gautheret, along with the late Philip R. White and P. Nobécourt, played a key pioneering and decisive role in the development of the modern science of plant cell and tissue culture. This chapter is followed by others on callus and cell suspension cultures, nutrition and photoautotrophic growth, cytodifferentiation, and cryopreservation. These accounts of the basic and fundamental aspects of plant cell cultures prepare the ground for the next volume.

I thank all the authors for their fine contributions and members of the Editorial Advisory Board for their suggestions and support. It is also a pleasure to thank the editorial staff of Academic Press for their assistance in the preparation of this volume.

Indra K. Vasil

Contents

CHAPTER 1

History of Plant Tissue and Cell Culture: A Personal Account

R. J. Gautheret

Paris, France

I. INTRODUCTION

The success of experimental research is subject to two important factors. One is the outcome of unexpected discoveries and the other results from technical advances. For example, the wide implications of radioactivity and immunology were discovered by casual observations that significantly and fortunately were perceived by scientists of genius. On the other hand, the invention of the electron microscope revolutionized the study of cytology, whereas the availability of radioisotopes allowed the fantastic boom of biochemistry.

The development of the science of tissue culture was more complicated. Interest in it had been foreseen before the middle of the nineteenth century by the promoters of the cell theory. One experimental approach was unsuccessfully attempted during the first years of the twentieth century, but success was reached in only 1912 with animal cells, and another 22 years later with plant tissues.

The technique of cell culture was exploited quickly for animal cells, while in the case of plant tissue culture a long period of stagnation followed the initial establishment of the basic methods. After 30 years of relative indifference, thousands of scientists rushed suddenly toward this "new field" of plant biology, which currently is undergoing considerable expansion with the new name of biotechnology.

II. PREHISTORY OF TISSUE CULTURE

A. Discovery of Callus Formation

The prehistory of plant tissue culture began more than 225 years ago. The discovery of callus formation was made by Duhamel, "Seigneur du Monceau et de Vrigny" (Fig. 1). Like many of the bright people of the eighteenth century, he was an encyclopedist. He published 11 volumes on naval architecture and 18 of a dictionary of sciences and arts. In the manor of his brother, which was located close to Pithiviers (about 50 miles from Paris), he made many observations and experiments on plants, especially on trees. His results were published in two treatises: the dictionary of trees and bushes, and the well-known "La Physique des Arbres" (Duhamel du Monceau, 1756). In this latter book, he described many experiments on sap circulation, grafting, and wound healing. Removing a small ring of cortex from an elm, he observed the development of a swelling above the area of

Fig. 1. Henri-Louis Duhamel du Monceau (1700–1782). General Inspector of the Navy and an agronomist. His pioneering experiments on wound healing identified in 1756 the plant material that was used 178 years later for the first successful tissue cultures.

decortication, while buds developed on the lower part (Fig. 2). Duhamel du Monceau noticed that the buds seemed to come out from the interface between wood and cortex. He had the prescience of cambium when he described "un tissu cellulaire très abreuvé et très délié qui, quand il sera converti en bois, unira l'une a l'autre deux couches très minces de fibres longitudinales" (Duhamel du Monceau, 1756). This very old work was a foreword for the discovery of plant tissue culture. But in 1756 the bacteriological technique was not invented, asepsis was unknown, the concept of tissue culture had not yet been expressed, and finally nobody was able to appreciate Duhamel's discovery.

B. The Cell Theory

About 160 years after the extensive work of Malpighi (1675) on microscopic anatomy, the celebrated cell theory was expressed independently and almost simultaneously by Schleiden (1838) with respect to plants and

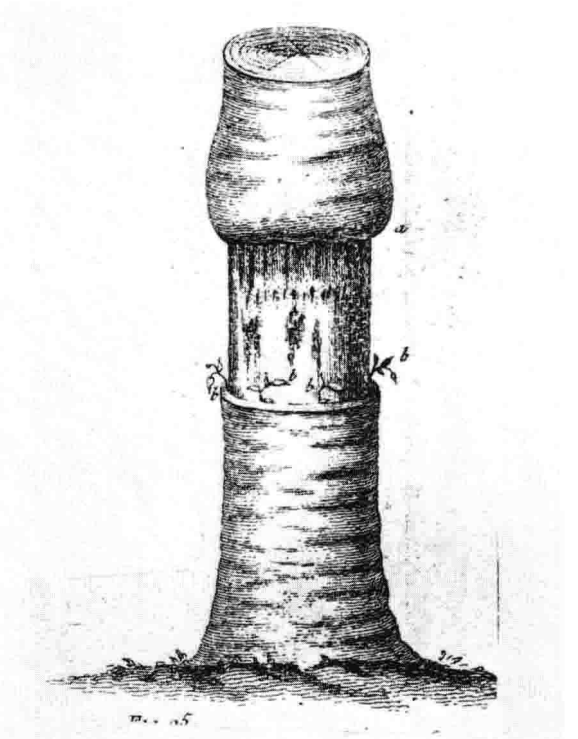

Fig. 2. Wound healing following ring-shaped "debarking" of an elm tree. A swelling developed above the debarked area, while buds appeared on the lower part (Duhamel du Monceau).

by Schwann (1839) considering both animals and plants. In their publications, these two biologists admitted implicitly that the cell is capable of autonomy and even that it is totipotent. This was expressed very clearly by Schwann (1839) in these words:

> Among the lower plants, any cell can be separated from the plant and continue to grow. Thus, entire plants may consist of cells whose capacity for independent life can be clearly demonstrated. . . . That not every cell, when separated from the organism, does in fact grow is no more an argument against this theory than is the fact that a bee soon dies when separated from the swarm a valid argument against the individual life of bee [translated from German].

In the case of egg cells of all organisms and spores of haplobiontic plants which are able to divide and form complete individuals, totipotency is obvious. This totipotent behavior occurs similarly in the animal kingdom, for example when a somatic cell of a hydra gives rise to a new individual, and in the case of some plants such as *Begonia*, whose epidermal cells may transform into new begonias (Hartsema, 1926). But in many cases, somatic

cells do not produce complete organisms. Their totipotency was postulated by the cell theory, but neither Schleiden nor Schwann proposed any experimental methods capable of proving this consequence of their theory. This limitation of the cell theory was of course distinctly perceived by Schwann (see the previous quotation). The demonstration required two steps: first, the multiplication of a single cell and, second, its trasformation into a complete organism. The first step was reached in 1954 and the second 11 years later. However, this ultimate target was attained by a very long and often illogical, but very interesting, journey.

While Duhamel du Monceau had shown the way, Schleiden and Schwann defined the aim. The race began slowly and initially by the avenue opened by Duhamel. Trécul (1853) performed experiments on callus formation by decorticated trees such as *Robinia, Pawlonia, Ulmus,* and others. While Duhamel had almost neglected the microscope, Trécul used it intensively and published a large number of excellent pictures of callus sections. These proved that scar tissue comes not only from cambium but also from the phloem, and even from medullary rays and the youngest parts of the xylem (Fig. 3). He had really shown what material could lead to tissue culture.

Similar experiments were undertaken by Vöchting (1878). He obtained very luxuriant callus, for example, with explants from *Brassica rapa.* His attention was especially attracted by the polarity that characterizes the development of plant fragments. Thus, the upper portion of a piece of stem always produced buds, while the basal portion produced callus or roots. Even very thin slices showed such a polar development. Thus, this property belongs to the cell itself.

Other experiments carried out by Goebel (1902) confirmed Vöchting's results and supported the notion of totipotency. Vöchting undertook grafting experiments between different species of *Opuntia, Salix, Beta,* and others. These experiments demonstrated that the behavior of a tissue is not altered by contact with another tissue. The dependence of morphogenetic capacity on hereditary internal factors is very strict. A synthetic view of the results provided by experimental morphology led Sachs (1880–1882) to conceive a general theory that suggested the existence of organ-forming substances distributed in a polar fashion. A similar view was proposed by Wiesner (1884) from experiments on roots.

Nine years later, a new approach to tissue culture was used by Rechinger (1893) when he determined the minimal size of explants which permits cell division. For this purpose, he isolated small pieces from buds of *Populus nigra* and *Fraxinus ornus,* from roots of *Beta vulgaris* and *Brassica rapa,* and from stems of *Pothos celatocaulis* and *Coleus arifolia.* The explants were placed at the surface of moistened sand. Pieces thicker than 20 mm were sometimes able to produce buds and even to regenerate entire plants.