PLANT TISSUE CULTURE AND ITS AGRICULTURAL APPLICATIONS

LYNDSEY A WITHERS
PG ALDERSON

BUTTERWORTHS

Plant Tissue Culture and its Agricultural Applications

LYNDSEY A. WITHERS
P. G. ALDERSON
University of Nottingham School of Agriculture

BUTTERWORTHS
London Boston Durban Singapore Sydney Toronto Wellington

First published 1986

British Library Cataloguing in Publication Data

Plant tissue culture and its agricultural applications.
1. Plant tissue culture 2. Plant propagation
I. Withers, Lyndsey A. II. Alderson, P.G.
631.5'2 SB123.6

ISBN 0-407-00921-3

Library of Congress Cataloging in Publication Data
Main entry under title:

Plant tissue culture and its agricultural applications.

Includes indexes.
1. Plant propagation—In vitro—Congresses.
2. Plant tissue culture—Congresses. 3. Germplasm resources, Plant—Storage—In vitro—Congresses.
4. Plant genetic engineering—Congresses. 5. Plant-breeding—Congresses. 6. Crops—Congresses.
I. Withers, Lyndsey A. II. Alderson, P.G.
SB123.6.P519 1986 631.5'3 85-22425

ISBN 0-407-00921-3

Photoset by Scribe Design, Gillingham, Kent
Printed in Great Britain at the University Press, Cambridge

PREFACE

Plant tissue culture techniques have introduced a new phase into agriculture, following on from the 'green revolution'. In view of this development and of the potential impact of *in vitro* technology in all aspects of pure and applied crop research, the 41st Easter School was planned around the theme of 'The Tissue Culture Revolution'. While in some ways this might seem a fanciful title, all revolutions involve a great deal of 'back-room' work which enables more dramatic advances to take place. In the case of plant tissue culture research, many individually small increments in the store of knowledge make possible the progress that results in improvements in crop production in the field. In recognition of this fact, a number of the chapters in this volume are of a review nature and present current knowledge in particular areas, whilst others describe recent research findings pertinent to the preceding review topic.

The elements of the tissue culture revolution can be identified as the mass propagation of elite plant material, its improvement in the phytopathological sense, its conservation in a stable, healthy form, and the development of new, improved genotypes. The sessions of the Easter School and the sections of this volume reflect these areas. As tissue culture is a broad, interdisciplinary subject, whose practitioners have wide ranging expertise and experience, there are inevitable instances of overlap, giving difficulties in the most appropriate location of a particular piece of work. This is a testimony to its complex, integrated nature rather than an indication of a lack of clarity in the evaluation of the subject. Thus, good *in vitro* conservation requires the development of competence in clonal propagation, as does capitalization upon the development of methods of genetic manipulation. Accordingly, the reader is encouraged to examine all sections of the volume for aspects of interest and relevance.

The core of this volume is embraced by introductory and final chapters which aim to interrelate the different subject areas, place research progress into context, and provide a realistic evaluation of the future research emphases required if the tissue culture revolution is to come to full fruition. The final chapter also includes pertinent points from the poster sessions presented at the conference and a digest of the extensive discussions held both formally and informally between delegates. These discussions were targeted towards areas currently perceived as presenting particular impediments to progress.

The editors are indebted to the many contributors to this volume for their hard work and cooperation in presenting both their spoken papers and their manuscripts. The successful organization of the conference owes much to the hard work and dedication of Mrs Jeanne Rodwell. In addition, we wish to thank our students and colleagues and the Session Chairmen whose efforts contributed to the smooth running of the conference. Grateful thanks are due to the companies listed in the Acknowledgements whose financial support made possible the participation of a number of the conference delegates.

Lyndsey A. Withers and P.G. Alderson

ACKNOWLEDGEMENTS

Donations are acknowledged from the following:

Elsevier Science Publishers
Flow Laboratories Ltd
Imperial Chemical Industries plc (Plant Protection Division)
L.H. Fermentation Ltd
Laboratory and Electrical Engineering Co. Ltd (LEEC)
E. Leitz (Instruments) Ltd
Longman Group Ltd
Marks and Spencer plc
Microflow, Dent and Hellyer (MDH) Ltd
Microplants
Munton and Fison plc
Nickerson RPB Ltd
Oxford University Press
Planer Products Ltd
Sigma Chemical Company Ltd

EDITORS' NOTE

The editors have not attempted to unify all units in the various chapters. In some cases this has been to avoid unhelpful conversions and in others because no direct conversions could be made, as in the case of light measurements. (Further information and discussion of the latter may be found in a paper by J.S. McLaren (1980). The expression of light measurements in relation to crop research. In *Seed Production*, P.D. Hebblethwaite, Ed., 28th Easter School in Agricultural Science, Butterworth, London, pp. 663–670.)

For the reader's guidance, the following molecular weights are given to permit conversion between molar and by weight expressions of concentrations of the most common culture medium additives.

Sucrose	342.3
Mannitol	182.2
Inositol	180.2
Naphthaleneacetic acid (NAA)	186.2
Indoleacetic acid (IAA)	175.2
Indolebutyric acid (IBA)	203.2
2,4-Dichlorophenoxyacetic acid (2,4-D)	221.0
6-Benzylaminopurine (BAP)	225.2
Kinetin	215.2
Zeatin	219.3
Isopentyladenine (2iP)	203.3
Gibberellic acid (GA_3)	346.4
Abscisic acid (ABA)	264.3

CONTENTS

I

Setting the scene

1

THE TISSUE CULTURE REVOLUTION

E.C. COCKING
Plant Genetic Manipulation Group, University of Nottingham, Nottingham, UK

Introduction

The present tissue culture revolution stems largely from the fact that plant tissue culture, and associated cloning techniques, provide the foundation for the exploitation of genetic engineering. Tissue culture propagation is also a way to study the mechanisms by which cells differentiate, thereby providing an experimental approach to link genotype with phenotype. As a consequence, there is an intense general interest in plant tissue culture, which has been reflected in numerous symposia and reviews. Moreover, the discussion of the uses of tissue culture for the future of crops has become a popular feature for many periodicals.

As discussed by Roberts (1982), the task of the developmental biologist, pursuing the regeneration of plants from culture, is not an easy one; the trick in achieving regeneration seems to be how to trigger the gene segments that initiate and control differentiation and development. Yet such fundamental scientific enquiry has been largely ignored in the tissue culture research of the past twenty years. As will be discussed later, DNA transposition may be the mechanism by which cells become committed to different fates during cellular differentiation. That the regulation of plant genes is more complex than that of bacterial genes is an additional complicating factor.

Plant tissue culture permeates plant biotechnology and cements together its various aspects; to a large extent the tissue culture revolution has occurred *because* of the needs of this new plant biotechnology.

The historical groundwork

EARLY PROBLEMS AND PROMISE

Probably the first step towards a technique for the cultivation of tissues outside the body of an organism was a realization of the importance of the 'internal environment', not merely as something produced in metabolism, but also as a medium in which the activities of the tissues could be regulated. The first experimental step was made by Roux (1885) when he removed a fragment of the neural plate of a chick embryo and cultivated it in a warm salt solution. Although as

discussed by Krikorian and Berquam (1969), real success first came with animal tissue, the botanist Gottlieb Haberlandt (1854–1945) clearly set forth the purposes and potentialities of cell culture after having attempted, and failed in, the culture of isolated plant cells. As recounted by Dawes (1952) these tentative methods gave way to a more definite and reliable technique after Harrison (1907) devised methods of cultivating fragments of living nerve, a technique greatly improved by Alexis Carrel. The first demonstration by Carrel, in Paris in 1910, of the cultivation of cells outside the body of the organism was somewhat sensational whereupon well-known biologists declared his tissue fragments to show marked signs of necrosis. This did not deter Carrel who persevered with the technique and who was able, in 1948, to state succinctly that 'owing to the new procedures for the cultivation of tissues, it has been possible to study living cells in a flask as easily as bees in a hive' (Carrel, 1948).

To some extent, similar difficulties confronted Haberlandt but, unlike Carrel, Haberlandt did not pursue his cell culture studies. Moreover, he used three monocotyledonous genera for much of his work, and green mesophyll and palisade cells that were mature, highly differentiated and also sometimes contaminated with bacteria. Even if he was working aseptically on these cultures today and used improved culture media, it is unlikely that he would achieve sustained division of these particular cells from these particular species. Mature, highly differentiated cells are more difficult to culture than meristematic cells and, for the most part, monocotyledonous plants only yield vigorously growing cells, tissue and organ cultures with difficulty (Krikorian and Berquam, 1969).

It was not until 1957 that kinetin was discovered and the idea introduced (for tobacco) of synergistic effects of auxins and cytokinins in promoting cell division (Skoog and Miller, 1957). Indeed, Haberlandt[a] himself, in concluding his seminal paper (1902) on *Experiments on the Culture of Isolated Plant Cells* says: 'I should like to point out the fact that, in my cultures, despite the conspicious growth of the cells which frequently occurred, cell division was never observed. It will be the problem of future culture experiments to discover the conditions under which isolated cells undergo division.' How right he was!

A NEW APPROACH WITH CALLUS AND MERISTEMS

Gautheret (1983), in his recent survey of the history of plant tissue culture, has commented that Haberlandt seems to have been obsessed by the cellular theory and did not suspect that experiments on callus could be a step toward tissue and even cell cultures. Indeed, while Carrel and his collaborators were making great strides with animal tissues, investigations in plant tissue were orientated for the first quarter of this century by the profound influence of Haberlandt. Experiments on the culture of isolated cells by numerous other investigators were also unsuccessful. As a result of their failed attempts, different approaches were developed. These led to successful root-tip culture, demonstration of totipotency and, ultimately, the division of isolated cells in culture coupled with whole plant regeneration. Kotte (1922) succeeded in cultivating small root tips of pea and maize in various nutrients. The roots developed well and their growth was maintained for long periods, but no

[a]See Krikorian and Berquam (1969) for a full translation of this paper (Haberlandt, 1902) from the original German.