HANDBOOK of PLANT CELL CULTURE

VOLUME 4

Techniques and Applications

Edited by

D. A. Evans, W. R. Sharp,
P. V. Ammirato

HANDBOOK OF PLANT CELL CULTURE,
Volume 4

Techniques and Applications

Editors

David A. Evans
DNA Plant Technology Corporation

William R. Sharp
DNA Plant Technology Corporation

Philip V. Ammirato
Barnard College, Columbia University
DNA Plant Technology Corporation

Macmillan Publishing Company
NEW YORK

Collier Macmillan Publishers
LONDON

Macmillan Publishing Company
866 Third Avenue, New York, NY 10022

Collier Macmillan Canada, Inc.

Printed in the United States of America

printing number
1 2 3 4 5 6 7 8 9 10 year 6 8 7 8 9 0 1 2 3 4

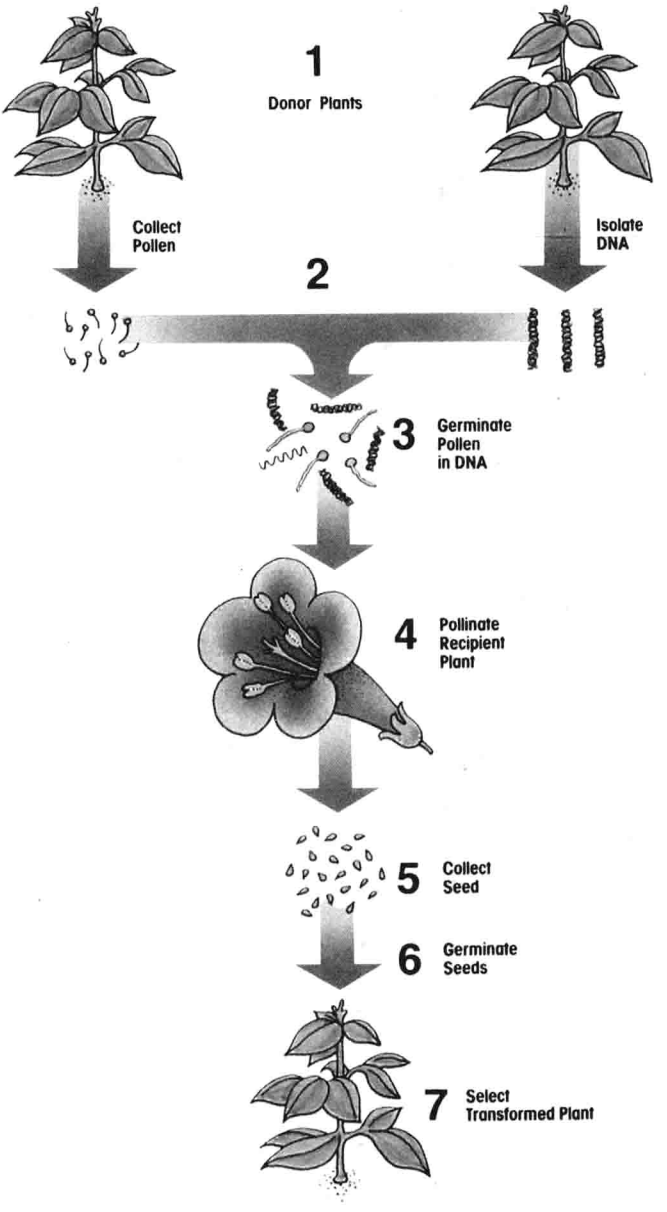
Library of Congress Cataloging-in-Publication Data

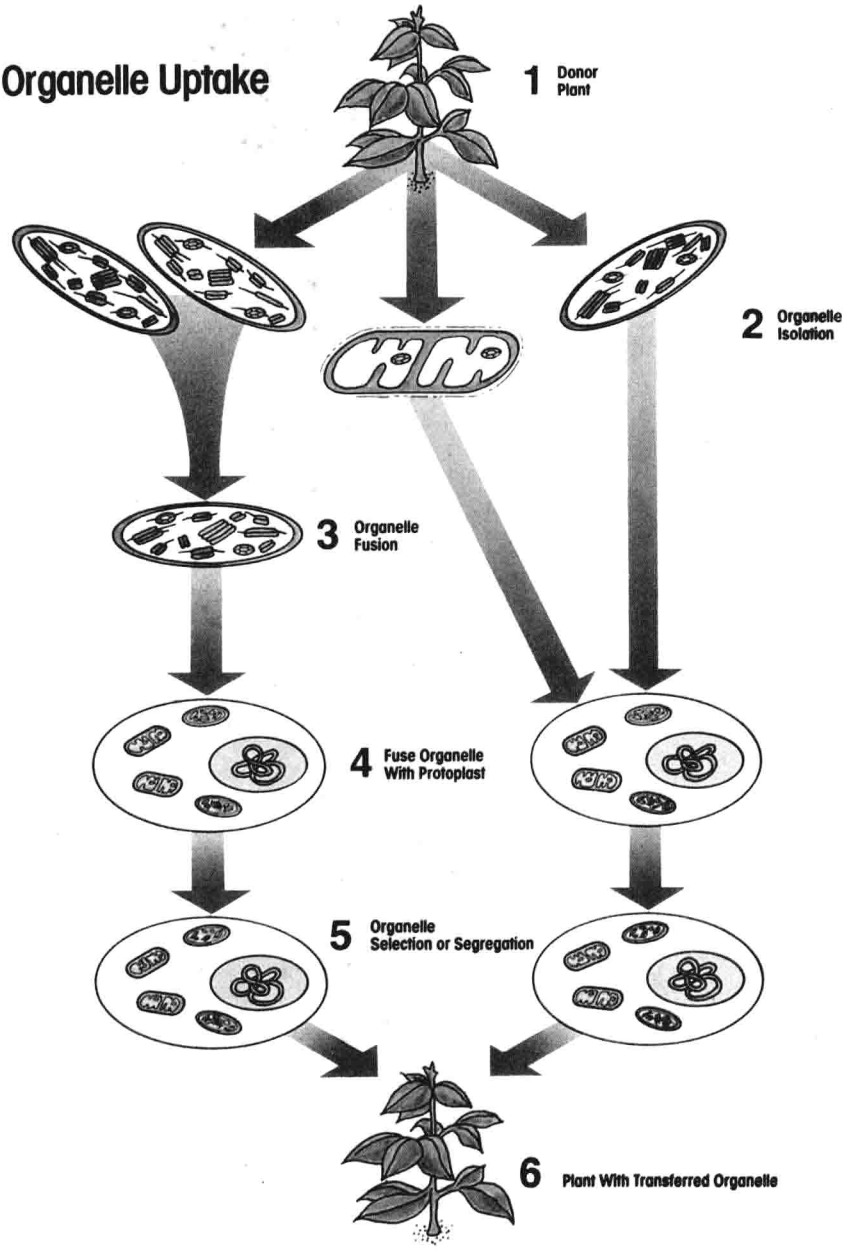
(Revised for vol. 4)
Main entry under title:

Handbook of plant cell culture.

Edited by David A. Evans and others.
Includes bibliographies and indexes.
Contents: v. 1. Techniques for propagation and breeding—v. 2. Crop species—[etc.]—v. 4. Techniques and application.
1. Plant cell culture—Handbooks, manuals, etc.
I. Evans, David, 1952– . II. Sharp, William R., 1936– .
III. Ammirato, Philip V., 1943– .
SB123.6.H36 1983 631.5'2 82-73774
ISBN 0-02-949230-0 (v. 1)

DNA Uptake Using Germinated Pollen





ABBREVIATIONS

Growth Regulators

IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
NAA	1-Naphthaleneacetic acid
2,4-D	(2,4-Dichlorophenoxy)acetic acid
2,4,5-T	(2,4,5-Trichlorophenoxy)acetic acid
CPA	(4-Chlorophenoxy)acetic acid
PIC	Picloram (4-amino-3,5,6-trichloropicolinic acid)
NOA	2-Naphthoxyacetic acid
BTOA	2-Benzothiazoleacetic acid
BA or 6BA	6-Benzylaminopurine
ZEA	Zeatin
KIN	Kinetin
2iP	(2-Isopentenyl)adenine

Additives

CH	Casein hydrolysate
CW	Coconut water
EDTA	(Ethylenedinitrilo)tetraacetic acid
GA	Gibberellic acid (Gibberellin A_3)
ABA	Abscisic acid

Macro- and Micronutrient Formulations

ADE	Adenine
MS	Murashige and Skoog (1962)
B5	Gamborg et al. (1968)
ER	Erikkson (1965)
WH	White (1963)
SH	Schenk and Hildebrandt (1972)

Formulations of Culture Media Used Most Often for Plant Cell Culture

MACRO-NUTRIENTS	MS		B5		WH[a]	
	mM	mg/l	mM	mg/l	mM	mg/l
NH_4NO_3	20.6	1650	-	-	-	-
KNO_3	18.8	1900	25	2500	0.8	80
$CaCl_2 \cdot 2H_2O$	3.0	440	1.0	150	-	-
$MgSO_4 \cdot 7H_2O$	1.5	370	1.0	250	3.0	737
KH_2PO_4	1.25	170	-	-	-	-
$(NH_4)_2SO_4$	-	-	1.0	134	-	-
$NaH_2PO_4 \cdot H_2O$	-	-	1.1	150	0.12	19
$Ca(NO_3)_2 \cdot 4H_2O$	-	-	-	-	1.2	288
KCl	-	-	-	-	0.9	65
Na_2SO_4	-	-	-	-	1.4	200

MICRO-NUTRIENTS	MS		B5		WH[a]	
	µM	mg/l	µM	mg/l	µM	mg/l
KI	5	0.83	4.5	0.75	4.5	0.75
H_3BO_3	100	6.3	50	3.0	(25)	(1.5)
$MnSO_4 \cdot 4H_2O$	100	22.3	-	-	29.8	6.65
$MnSO_4 \cdot H_2O$	-	-	60	10	-	-
$ZnSO_4 \cdot 7H_2O$	30	8.6	7	2.0	9.3	2.67
$Na_2MoO_4 \cdot 2H_2O$	1.0	0.25	1.0	0.25	-	-
MoO_3	-	-	-	-	(0.007)	(0.0001)
$CuSO_4 \cdot 5H_2O$	0.1	0.025	0.1	0.025	(0.004)	(0.001)
$CoSO_4 \cdot 6H_2O$	0.1	0.025	0.1	0.025	-	-
$Fe_2(SO_4)_3$	-	-	-	-	6.3	2.5
Na_2EDTA	100	37.3	100	37.3	-	-
$FeSO_4 \cdot 7H_2O$	100	27.8	100	27.8	-	-

[a]Many inconsistencies have appeared in the literature regarding the basal salt composition first reported by White (1943). This formulation is based on a recent reevaluation (Singh, M. and Krikorian, A.D. 1981. White's standard nutrient solution. Ann. Bot. 47:133–139).

HANDBOOK OF PLANT CELL CULTURE

Published

Volume 1, *Techniques for Propagation and Breeding*
Editors: David A. Evans, William R. Sharp, Philip V. Ammirato, Yasuyuki Yamada

Volume 2, *Crop Species*
Editors: William R. Sharp, David A. Evans, Philip V. Ammirato, Yasuyuki Yamada

Volume 3, *Crop Species*
Editors: Philip V. Ammirato, David A. Evans, William R. Sharp, Yasuyuki Yamada

Volume 4, *Techniques and Applications*
Editors: David A. Evans, William R. Sharp, Philip V. Ammirato

Forthcoming

Volume 5, *Ornamental Species*
Editors: Philip V. Ammirato, David A. Evans, William R. Sharp, Yashpal P. S. Bajaj

Preface

Volume 4 begins the second phase of the *Handbook of Plant Cell Culture*, a series designed to bring together critical reviews of plant cell and tissue culture techniques and their application to specific crops. This treatise is consistent with earlier ones in providing reviews of topics that are particularly relevant to the current state of the art.

The science of plant cell culture, linking developmental, cellular, and molecular genetics with conventional plant breeding, enjoys a pivotal position in the general area of agricultural biotechnology, as seen in the commitments being made by major academic institutions, research foundations, and multinational corporations. Agricultural planners are depending on plant cell culture and the new genetics to quicken the pace of plant breeding and allow food production to meet the needs of a world population that will double, from 4 to 8 billion, in the next 50 to 60 years. Moreover, this new technology fits within the 3 to 5 year corporate research time frame of industry in allowing for the production of value-added consumer and industrial products with an agricultural base.

In Part B, devoted to techniques, a discussion of embryo culture emphasizes its role in circumventing some of the problems that confront the breeder using traditional methods. A second chapter discusses somaclonal variation and how it benefits a breeding program by speeding up the process of variety development. Other important techniques presented in this section include cellular selection methods for herbicide resistance, electrofusion for production of somatic cell

hybrids, organelle transfer, Ti-plasmid transformation, construction of wide hybrids in the cereals, and cell layer technology. Two other chapters pertain to plant-based secondary products and the immobilization of plant cells.

In Part C the application of plant cell culture techniques to the propagation and improvement of 11 crop species is discussed. These add to the 37 crops presented in the previous volumes and further extend the list of important crops, including cereals, legumes, vegetables, roots and tubers, temperate and tropical fruits, and those that provide fiber and wood as sources of extractable products.

The chapters on techniques and crop species are organized using the same format of the previous volumes. As before, the centerpiece for the volume is practical methodology, and we have again included in each chapter a major section of actual protocols, whether as recipes, tables, charts, or narratives.

In Part A we are pleased to have two introductory essays by distinguished scientists. Norman Borlaug, a pioneer in the use of shuttle breeding to develop improved wheat varieties, provides an overview of technology as applied to plant breeding and crop production. Dr. Borlaug was awarded the 1970 Nobel Peace Prize for his scientific efforts in bringing about the "Green Revolution." In his essay Dr. Borlaug discusses the realities pertaining to the movement of new technology into a commercial environment and the need to put plant cell culture and the new genetics in operation much faster than has been done with the technologies that contribute to conventional breeding. He points out the financial need to develop appropriate linkages between agricultural biotechnology and the financial sectors in order to make major quantitative and qualitative agricultural breakthroughs happen, and he places responsibility on scientists to work with plants of economic importance to quicken the pace for movement of science from the laboratory into the field.

A second essay provides highlights of the scientific career of Armin Braun of the Rockefeller University. His career has been unique in that he is a pioneer in the application of plant cell culture to the study of a practical problem. Dr. Braun focuses in his essay on the problems of plant cancer, with particular emphasis on the crown gall tumors caused by the tumor-forming bacterium, *Agrobacterium tumefaciens*. This research has shed new light on the developmental genetics of plant tumor development and on the more general problem of cancer itself. It is important to note that the Ti plasmid, the active agent associated with the bacterium, ushered in the era of gene transfer and splicing in higher plants. This technology is one of the key techniques under the umbrella of agricultural biotechnology.

Our readership has encouraged the preparation of additional volumes to provide updates on techniques as well as expand on the application of these techniques to the improvement of agronomic crops not covered in the first three volumes. It is our hope that Volume 4, and subsequent volumes, will continue to be of value to the plant cell culture scientist. We have tried to be responsive to the published reviews and to the many letters and telephone calls. This in-

formation has been invaluable in our continuing efforts to provide high standards for the series. We continue to encourage your comments on the subject matter and its organization for future volumes in this series.

As the *Handbook* moves into this second phase, we are indebted to the ongoing efforts of our editors at Macmillan, Sarah Greene and Frances Tindall. Their expertise and encouragement is sincerely appreciated. Special thanks, also, to Janis Bravo, who continues as editorial assistant, a position she fills with good grace, common sense, and uncommon organization. We also thank our typists, Karen Selover and JoAnn Morrison, and indexer, Mary Ellen Curtin. Lastly, we give especial thanks to our many contributors, an international group of scientists dedicated to the rapid communication of science.

Contributors

M. R. Ahuja	Federal Research Center for Forestry and Forest Products, Institute of Forest Genetics and Forest Tree Breeding, Grosshansdorf, West Germany
P. V. Ammirato	Department of Biology, Barnard College, Columbia University, New York, NY and DNA Plant Technology Corporation, Cinnaminson, NJ
A. I. Atanassov	Tissue Culture Laboratory, Institute of Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria
N. E. Borlaug	CIMMYT, El Batan, Mexico
D. S. Brar	Department of Plant Breeding, Punjab Agricultural University, Ludhiana, Punjab, India
A. C. Braun	Ridgeview Road, Princeton, NJ
P. Brodelius	Institute of Biotechnology, Swiss Federal Institute of Technology, Zurich, Switzerland
R. S. Chaleff	Department of Central Research and Development, Experimental Station, E.I. DuPont deNemours and Company, Wilmington, DE
J. Doležel	Czechoslovak Academy of Sciences, Institute of Experimental Botany, Olomouc, Czechoslovakia
J. M. Dunwell	John Innes Institute, Norwich, England
J. A. Eash	Department of Vegetable Crops, University of California, Davis, CA
D. A. Evans	DNA Plant Technology Corporation, Cinnaminson, NJ

B. V. Gantotti	Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan, Canada
J. J. Gaynor	Department of Botany, Rutgers University, Newark, NJ
S. Gleddie	Ottawa Research Station, Agriculture Canada, Ottawa, Canada
T. Hashimoto	Research Center for Cell and Tissue Culture, Kyoto University, Kyoto, Japan
L. Havel	Czechoslovak Academy of Sciences, Institute of Experimental Botany, Olomouc, Czechoslovakia
C. Y. Hu	Biology Department, William Paterson College of New Jersey, Wayne, NJ
K. K. Kartha	Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan, Canada
G. S. Khush	Plant Breeding Department, International Rice Institute, Manila, Philippines
R. E. Koning	Department of Botany and Plant Physiology, Rutgers University, Piscataway, NJ
W. A. Keller	Ottawa Research Station, Agriculture Canada, Ottawa, Ontario, Canada
F. A. Krens	Department of Plant Molecular Biology, State University of Leiden, Leiden, The Netherlands
R. W. Litz	Tropical Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, Homestead, FL
R. W. Michelmore	Department of Vegetable Crops, University of California, Davis, CA
R. A. Morrison	DNA Plant Technology Corporation, Cinnaminson, NJ
F. J. Novák	Czechoslovak Academy of Sciences, Institute of Experimental Botany, Olomouc, Czechoslovakia
E. Rugini	Center for the Study of Olive Culture, Perugia, Italy
R. A. Schilperoort	Department of Plant Molecular Biology, State University of Leiden, Leiden, The Netherlands
G. Setterfield	Department of Biology, Carleton University, Ottawa, Ontario, Canada
W. R. Sharp	DNA Plant Technology Corporation, Cinnaminson, NJ
K. Tran Thanh Van	The Phytotron, National Center for Scientific Research, Gif sur Yvette, France
T. H. Trinh	The Phytotron, National Center for Scientific Research, Gif sur Yvette, France
P. Wang	Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China
R. J. Whitaker	DNA Plant Technology Corporation, Cinnaminson, NJ
G. J. Wullems	Department of Plant Molecular Biology, State University of Leiden, Leiden, The Netherlands

Bud Grace in *Science '85*

HANDBOOK OF PLANT CELL CULTURE

Contents