

Molecular Methods of Plant Analysis

Editors:

J.F. Jackson (Managing Editor)

H.F. Linskens

R.B. Inman

Volume 23

*Volumes Already Published in this Series
(formerly Modern Methods of Plant Analysis):*

- Volume 1:* Cell Components
1985, ISBN 3-540-15822-7
- Volume 2:* Nuclear Magnetic Resonance
1986, ISBN 3-540-15910-X
- Volume 3:* Gas Chromatography/Mass Spectrometry
1986, ISBN 3-540-15911-8
- Volume 4:* Immunology in Plant Sciences
1986, ISBN 3-540-16842-7
- Volume 5:* High Performance Liquid Chromatography in Plant Sciences
1986, ISBN 3-540-17243-2
- Volume 6:* Wine Analysis
1988, ISBN 3-540-18819-3
- Volume 7:* Beer Analysis
1988, ISBN 3-540-18308-6
- Volume 8:* Analysis of Nonalcoholic Beverages
1988, ISBN 3-540-18820-7
- Volume 9:* Gases in Plant and Microbial Cells
1989, ISBN 3-540-18821-5
- Volume 10:* Plant Fibers
1989, ISBN 3-540-18822-3
- Volume 11:* Physical Methods in Plant Sciences
1990, ISBN 3-540-50332-3
- Volume 12:* Essential Oils and Waxes
1991, ISBN 3-540-51915-7
- Volume 13:* Plant Toxin Analysis
1992, ISBN 3-540-52328-6
- Volume 14:* Seed Analysis
1992, ISBN 3-540-52737-0
- Volume 15:* Alkaloids
1994, ISBN 3-540-52738-9
- Volume 16:* Vegetables and Vegetable Products
1994, ISBN 3-540-55843-8
- Volume 17:* Plant Cell Wall Analysis
1996, ISBN 3-540-59406-X
- Volume 18:* Fruit Analysis
1995, ISBN 3-540-59118-4
- Volume 19:* Plant Volatile Analysis
1997, ISBN 3-540-61589-X
- Volume 20:* Analysis of Plant Waste Materials
1999, ISBN 3-540-64669-8
- Volume 21:* Analysis of Taste and Aroma
2002, ISBN 3-540-41753-2
- Volume 22:* Testing for Genetic Manipulation in Plants
2002, ISBN 3-540-43153-5
- Volume 23:* Genetic Transformation of Plants
2003, ISBN 3-540-00292-8

Genetic Transformation of Plants

Edited by
J.F. Jackson and H.F. Linskens

With 21 Figures, 4 in Color
and 11 Tables



Springer

Professor J.F. JACKSON
Department of Horticulture
Viticulture and Oenology
University of Adelaide
Waite Campus
SA 5064 Glen Osmond
Australia

Professor H.F. LINSKENS
Goldberglein 7

91056 Erlangen
Germany

Professor R.B. INMAN
Institute of Molecular Virology
University of Wisconsin-Madison
Robert M. Bock Laboratories
1525 Linden Drive
Madison, Wisconsin 53706-1596
USA

ISSN 1619-5221

Cataloging-in-Publication Data applied for

A catalog record for this book is available from Library of Congress.
Bibliographic information published by Die Deutsche Bibliothek.
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed
bibliographic data is available in the Internet at <http://dnb.ddb.de>

Library of Congress Cataloging-in-Publication Data

Genetic transformation of plants / edited by J.F. Jackson and H.F. Linskens.
p. cm. – (Molecular methods of plant analysis, ISSN 1619-5221 ; v. 23)
Includes bibliographical references and index.
ISBN 978-3-642-05553-9 ISBN 978-3-662-07424-4 (eBook)
DOI 10.1007/978-3-662-07424-4

1. Plant genetic transformation. 2. Crops–Genetic engineering. 3. Plant genetic
engineering. I. Jackson, J. F. (John F.), 1935– II. Linskens, H. F. (Hans F.), 1921– III. Series.

QK865.M57 vol. 23
[SB123.57]
571.2'028 s–dc21
[631.5'233]

2003042766

This work is subject to copyright. All rights reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broad-casting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag Berlin Heidelberg GmbH. Violations are liable for prosecution under the German Copyright Law.

<http://www.springer.de>

© Springer-Verlag Berlin Heidelberg 2003

Originally published by Springer-Verlag Berlin Heidelberg New York in 2003
Softcover reprint of the hardcover 1st edition 2003

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Production: PRO EDIT GmbH, Heidelberg

Cover design: design & production GmbH, Heidelberg
Cover photograph: Dr. Malin Elfstrand and Mr Hartmut Weichelt
Typesetting: SNP Best-set Typesetter Ltd., Hong Kong
Printed on acid-free paper 31/3150 Di – 5 4 3 2 1 0

Preface

Molecular Methods of Plant Analysis

Concept of the Series

The powerful recombinant DNA technology and related developments have had an enormous impact on molecular biology. Any treatment of plant analysis must make use of these new methods. Developments have been so fast and the methods so powerful that the editors of *Modern Methods of Plant Analysis* have now decided to rename the series *Molecular Methods of Plant Analysis*. This will not change the general aims of the series, but best describes the thrust and content of the series as we go forward into the new millennium. This does not mean that all chapters a priori deal only with the methods of molecular biology, but rather that these methods are to be found in many chapters together with the more traditional methods of analysis which have seen recent advances. The numbering of the volumes of the series therefore continues on from 20, which is the most recently published volume under the title *Modern Methods of Plant Analysis*.

As indicated for previous volumes, the methods to be found in *Molecular Methods of Plant Analysis* are described critically, with hints as to their limitations, references to original papers and authors being given, and the chapters written so that there is little need to consult other texts to carry out the methods of analysis described. All authors have been chosen because of their special experience in handling plant material and/or their expertise with the methods described. The volumes of the series published up to now fall into three groups: Volumes 1–5 and Volume 11 dealing with some basic principles of methods, Volumes 6, 7, 8, 10, 14, 16, 18 and 20 being a group determined by the raw plant material being analysed, and a third group comprising Volumes 9, 12, 13, 15, 17 and 19 which are separated from the other volumes in that the class of substances being analysed, is indicated in the volume title. Volume 21 and future volumes of *Molecular Methods of Plant Analysis* will continue in a similar vein but will include more chapters involved with the methods of molecular biology.

Development of the Series

The handbook, *Modern Methods of Plant Analysis*, was first introduced in 1954, and was immediately successful, seven volumes appearing between 1956 and 1964. This first series was initiated by Michael Tracey of Rothamsted and Karl Paech of Tübingen. The so-called *New Series of Modern Methods of Plant Analysis*, Volumes 1–20, began in 1985 and has been edited by Paech's successor, H.F. Linskens of Nijmegen, The Netherlands, and John F. Jackson of Adelaide, South Australia. These same editors have now teamed up with a third, Ross B. Inman of Madison, Wisconsin, USA, to produce the renamed series *Molecular Methods of Plant Analysis*. As before, the editors are convinced that there is a real need for a collection of reliable, up-to-date methods of plant analysis covering large areas of applied biology ranging from agricultural and horticultural enterprises to pharmaceutical and technical organizations concerned with material of plant origin.

Future volumes will include Various Aspects of Plant Genomics.

Volume 23: Genetic Transformation of Plants

This third volume in the molecular series deals with the topic of genetic transformation of plants. Most would view genetic transformation as a means of bringing about plant improvement, however, it can be a useful tool in analysing the function of plant genes. To this end, the present volume focuses on genetic transformation of a range of plants by a range of methods, a multiplicity of methods being necessary as some plants are more difficult to transform than others.

Since in genetic transformation we are dealing with biotechnological innovation, this volume begins with a chapter on "Biotechnology, Genetic Manipulation and Intellectual Property Rights". It is beyond dispute that property rights apply to the products of biological research, and there is no doubt that in the "developed" world DNA sequences and cells of plant or animal origin can be patented. This first chapter then explores these property rights, be they physical or intellectual, and how they effect the use to which the transformed plant is put and the right to reproduce it.

The following chapter describes the many methods used to carry out plant transformation, beginning with *Agrobacterium rhizogenes*-mediated transformation, which leads to "hairy root" syndrome. This is particularly useful in analysing the interaction between roots and soil organisms or chemical compounds. Thus, promoter-trapping strategies using hairy roots have been utilized to identify genes that form nodules, while hairy roots have also been used to study the interaction between roots and nematodes. Analysis of responses to such chemicals as fungicides, nematicides and herbicides can also utilize the hairy root condition. The next chapter deals with *Agrobacterium tumefaciens*-mediated transformation of whole plants of *Petunia hybrida*, in this case, by a

suspension of *Agrobacterium* cells applied directly to the flower stigma at pollination. *Allium* species have well developed sulphur and carbohydrate biochemical pathways which need to be thoroughly investigated. However, *Allium* has proven to be very difficult to transform; a chapter on *Allium* transformation is therefore included in the belief that it will assist in the analysis of these pathways and identify which are important for normal physiology and which are crucial for the unique nutraceutical qualities ascribed to garlic and onions. Similarly, barley has proven difficult to transform, thus an electroporation method is described in this volume for barley.

Sorghum, like barley, proved difficult to transform at first. A chapter therefore follows on transformation of sorghum using *Agrobacterium tumefaciens*. Polyethylene glycol (PEG) was amongst the first gene transfer systems to be used for successful integration of foreign genes into plant cells. A chapter is included in this volume describing effective production of transgenic sunflower by a PEG-mediated transformation system. However, a large number of sunflower protoplasts need to be used to ensure a significant number of transformed plants. Sunflower exhibits considerable sexual incompatibility between crop and wild species, which limits access to the genetic pool for gene analysis (or plant improvement), and so transformation provides an alternative approach. The last few chapters of this book deal with particle bombardment and WHISKERS-mediated methods of transformation. Norway spruce transformation can be carried out by particle bombardment of embryonic cultures or pollen; the method is important in developing better or new qualities of wood. The author also discusses the considerable problems associated with gene flow by pollen following spruce transformation. A chapter follows on WHISKERS transformation of embryonic maize suspension cultures leading to regeneration into fertile transgenic plants. A subsequent chapter deals with genetic transformation of soybean with biolistics. The latter involves bombardment of proliferative embryonic cultures with DNA coated on 1- μ m diameter particles followed by selection and plant regeneration. Both spruce and soybean biolistic transformation methods described above utilized gold particles coated with DNA, although tungsten particles were used in the past. It seems that tungsten, unlike gold, causes considerable DNA damage including DNA strand scissions and inhibition of cell differentiation; these and other genotoxic effects of tungsten particles are assessed in the final chapter.

J.F. JACKSON, H.F. LINSKENS, R.B. INMAN

Contents

1 Exclusive Rights in Life: Biotechnology, Genetic Manipulation,
and Intellectual Property Rights

E.R. GOLD

| | | |
|-------------|--|----|
| 1.1 | Introduction | 1 |
| 1.2 | Biotechnological Innovation | 2 |
| 1.2.1 | Physical Innovations | 3 |
| 1.2.1.1 | DNA and Protein Molecules | 3 |
| 1.2.1.2 | Cells | 3 |
| 1.2.1.3 | Whole Organisms | 4 |
| 1.2.2 | Information and Other Intangibles | 4 |
| 1.2.2.1 | DNA Sequences and Cells | 4 |
| 1.2.2.2 | Processes Using Biological Matter | 5 |
| 1.2.2.3 | Bioinformatics | 5 |
| 1.2.3 | Summary | 6 |
| 1.3 | Introduction to Intellectual Property Rights | 6 |
| 1.3.1 | Exclusive Rights vs. Rights to Things | 6 |
| 1.3.2 | Property and Intellectual Property Rights | 7 |
| 1.3.3 | Trade Secrets | 7 |
| 1.3.3.1 | Subject Matter | 8 |
| 1.3.3.2 | Requirements | 8 |
| 1.3.4 | Patents | 8 |
| 1.3.4.1 | Subject Matter | 9 |
| 1.3.4.1.1 | Invention vs. Discovery | 10 |
| 1.3.4.1.2 | Exclusions | 10 |
| 1.3.4.2 | Requirements | 11 |
| 1.3.4.2.1 | Substantive Criteria | 11 |
| 1.3.4.2.1.1 | Novelty | 12 |
| 1.3.4.2.1.2 | Inventive Step (Nonobviousness) | 12 |
| 1.3.4.2.1.3 | Industrial Application (Utility) | 12 |
| 1.3.4.2.2 | Procedural Criterion: Disclosure | 13 |
| 1.3.4.3 | Remedies | 14 |
| 1.3.5 | Copyright and Database Protection | 14 |
| 1.3.5.1 | Subject Matter | 15 |

| | | |
|---------|--|----|
| 1.3.5.2 | Requirements | 15 |
| 1.3.5.3 | Remedies | 16 |
| 1.3.6 | Plant Variety Protection | 16 |
| 1.3.6.1 | Subject Matter | 16 |
| 1.3.6.2 | Requirements | 17 |
| 1.4 | Challenges | 17 |
| 1.4.1 | Incentive vs. Access | 18 |
| 1.4.1.1 | Justification for Property Rights | 18 |
| 1.4.1.2 | Economic Reality | 19 |
| 1.4.2 | Fairness to Providers of Biological Matter | 20 |
| 1.4.2.1 | Rights to Biological Matter | 20 |
| 1.5 | Conclusion | 21 |
| | References | 21 |

2 *Agrobacterium rhizogenes*-Mediated Transformation of Plants

W. VAN DE VELDE, M. KARIMI, G. DEN HERDER, M. VAN MONTAGU,
M. HOLSTERS, and S. GOORMACHTIG

| | | |
|-------|--|----|
| 2.1 | Introduction | 23 |
| 2.2 | Aspects Influencing <i>A. rhizogenes</i> Transformation | |
| | Efficiency | 26 |
| 2.2.1 | Choice of <i>A. rhizogenes</i> Strain | 26 |
| 2.2.2 | Choice of Explant | 28 |
| 2.2.3 | Preparation of Bacterial Inoculum and Infection of Explants | 29 |
| 2.2.4 | Co-cultivation | 29 |
| 2.3 | Establishing the Transformed Nature of Hairy Roots | 31 |
| 2.4 | Cotransformation of Binary T-DNA | 31 |
| 2.5 | Propagation of Hairy Root Lines in Liquid Cultures | 33 |
| 2.5.1 | The Clonal Status of Hairy Roots | 33 |
| 2.5.2 | Stability of Long-Term Hairy Root Cultures | 34 |
| 2.6 | Regeneration of Plants from Hairy Roots | 34 |
| 2.7 | The Multi-Auto-Transformation (MAT) Vector System | 35 |
| 2.8 | Conclusions | 37 |
| | Protocol 1: Production of Transformed Hairy Roots | 37 |
| | Protocol 2: Plant Regeneration from Hairy Roots | 38 |
| | Protocol 3: Hairy Root Liquid Culture | 39 |
| | References | 39 |

3 Transformation of *Petunia hybrida* by the *Agrobacterium* Suspension Drop Method

S.J. WYLIE, D. TJOKROKUSUMO, and J.A. MCCOMB

| | | |
|-----|---------------------------------|----|
| 3.1 | Introduction | 45 |
| 3.2 | Transformation | 47 |
| 3.3 | Analysis of Transformants | 47 |

| | | |
|-------|---|----|
| 3.3.1 | Screening <i>Petunia</i> Seedlings for Herbicide Resistance | 47 |
| 3.3.2 | Transmission of Basta Resistance Phenotype to T ₂ Progeny | 48 |
| 3.3.3 | β-Glucuronidase Assay | 48 |
| 3.3.4 | DNA Analysis | 49 |
| 3.4 | Conclusion | 49 |
| | References | 49 |

4 Onion, Leek and Garlic Transformation by Co-cultivation with *Agrobacterium*

C.C. EADY

| | | |
|---------|--|----|
| 4.1 | Introduction | 53 |
| 4.1.1 | Current Applications of <i>Allium</i> Transformation Technology | 53 |
| 4.1.1.1 | Physiological Studies | 53 |
| 4.1.1.2 | Herbicide Resistance | 54 |
| 4.1.1.3 | Antimicrobial Resistance | 54 |
| 4.1.1.4 | Insect Resistance | 55 |
| 4.2 | Onion Transformation Protocols | 55 |
| 4.2.1 | Transformation Using Antibiotic and Visual Selection | 56 |
| 4.2.1.1 | Bacterial Strain and Plasmids | 56 |
| 4.2.1.2 | Transformation Procedure (Modified from Eady et al. 2000 | 56 |
| 4.2.2 | Transformation Using Herbicide Selection | 57 |
| 4.2.2.1 | Bacterial Strain and Plasmids | 58 |
| 4.2.2.2 | Transformation Procedure | 58 |
| 4.2.3 | Ex-Flasking and Growth in Containment | 58 |
| 4.2.4 | Transgene Detection | 59 |
| 4.2.5 | Transgene Expression and Stability | 60 |
| 4.2.5.1 | Visual Reporter Genes | 60 |
| 4.2.5.2 | Expression of Herbicide Resistance | 61 |
| 4.2.5.3 | Antisense Alliinase Gene Expression | 61 |
| 4.3 | Leek Transformation | 62 |
| 4.4 | Garlic Transformation Protocol | 63 |
| 4.4.1 | Bacterial Strain and Plasmids | 63 |
| 4.4.2 | Transformation Procedure | 63 |
| 4.5 | Concluding Remarks | 64 |
| | References | 65 |

5 Electroporation Transformation of Barley

F. GÜREL and N. GÖZÜKIRMIZI

| | | |
|-----|--|----|
| 5.1 | Introduction | 69 |
| 5.2 | Background of Electroporation Procedures | 71 |

| | | |
|-------|---|----|
| 5.2.1 | Pre- and Post-Electroporation Period | 71 |
| 5.2.2 | Electrical Variables | 73 |
| 5.3 | Culture and Electroporation of Barley Explants | 74 |
| 5.3.1 | Protoplasts | 74 |
| 5.3.2 | Microspores | 76 |
| 5.3.3 | Intact Tissues | 77 |
| | 5.3.3.1 Analysis and Inheritance of Transgenes in Electroporated Tissues | 81 |
| 5.4 | Conclusions and Future Perspectives | 83 |
| | References | 84 |

6 Sorghum Transformation

Z. ZHAO and D. TOMES

| | | |
|-------|--|-----|
| 6.1 | Introduction | 91 |
| 6.2 | Sorghum Transformation Process and Optimization | 92 |
| 6.2.1 | Plant Materials and Transformation Systems | 92 |
| 6.2.2 | Transformation Via Microprojectile Bombardment | 93 |
| 6.2.3 | <i>Agrobacterium</i> -Mediated Transformation | 94 |
| 6.3 | Analysis of Transgenic Plants and the Progeny | 96 |
| 6.3.1 | Molecular Analysis of T ₀ Plants | 97 |
| 6.3.2 | Foreign Gene Expression in T ₀ Plants | 98 |
| 6.3.3 | Genetic and Molecular Analysis of the Progeny | 99 |
| 6.4 | Marker-Free Sorghum Transgenic Plants | 99 |
| 6.4.1 | Importance of Marker-Free Transgenics in Sorghum | 100 |
| 6.4.2 | Methods to Eliminate Markers from Transgenic Plants | 100 |
| 6.4.3 | <i>Agrobacterium</i> 2 T-DNA Co-Transformation System | 101 |
| | References | 102 |

7 Transgenic Sunflower: PEG-Mediated Gene Transfer

P.C. BINSFELD

| | | |
|-------|---|-----|
| 7.1 | Introduction | 109 |
| 7.2 | Genetic Variability and Transgenic Breeding | 109 |
| 7.3 | Gene Transfer Systems | 110 |
| 7.3.1 | PEG-Mediated Gene Transfer | 111 |
| | 7.3.1.1 Short DNA Molecule Uptake | 111 |
| | 7.3.1.2 Large DNA Molecule Uptake | 112 |
| 7.4 | Plant Regeneration | 114 |
| 7.5 | General Analytical Considerations | 115 |
| 7.5.1 | Molecular Analysis | 115 |
| | 7.5.1.1 DNA Extraction | 116 |
| | 7.5.1.2 Southern Hybridization | 116 |
| | 7.5.1.3 Polymerase Chain Reaction | 116 |
| | 7.5.1.4 Random Amplified Polymorphic DNA | 117 |

| | | |
|---------|---|-----|
| 7.5.2 | Biochemical Analysis | 118 |
| 7.5.2.1 | Multiple Molecular Forms of Enzymes | 118 |
| 7.5.2.2 | Enzymatic Assay | 119 |
| 7.5.3 | Cytogenetic Analysis | 120 |
| 7.5.3.1 | Flow Cytometric Analysis | 120 |
| 7.5.3.2 | Mitotic and Meiotic Cell Analysis | 122 |
| 7.5.3.3 | In Situ Hybridization | 122 |
| 7.5.4 | Morphological Analysis | 123 |
| 7.6 | Conclusions and Future Perspectives | 124 |
| | References | 124 |

8 Transformation of Norway Spruce (*Picea abies*) by Particle Bombardment

D.H. CLAPHAM, H. HÄGGMAN, M. ELFSTRAND, T. ARONEN,
and S. VON ARNOLD

| | | |
|-------|--|-----|
| 8.1 | Introduction | 127 |
| 8.2 | Types of Particle Accelerator | 127 |
| 8.3 | Transformation of Embryogenic Cultures | 128 |
| 8.3.1 | Transient Expression in Embryogenic Cultures | 128 |
| 8.3.2 | Production of Stably Transformed Cell Cultures and Transgenic Plants | 129 |
| 8.3.3 | Stability of Transgene Expression | 131 |
| 8.3.4 | Trends in Transgenic Plant Production | 131 |
| 8.4 | Transformation of Pollen | 133 |
| 8.4.1 | The Reproductive Biology of Norway Spruce | 133 |
| 8.4.2 | Transient Expression in Pollen | 134 |
| 8.4.3 | Development of Controlled Pollination Techniques for Bombarded Pollen | 135 |
| 8.5 | Applications of Transgenic Norway Spruce in Research | 136 |
| 8.5.1 | Genes Regulating Embryogenesis | 136 |
| 8.5.2 | Genes with Similarity to Defense Genes | 137 |
| 8.6 | Prospects for Transgenic Norway Spruce in Practical Forestry | 139 |
| | References | 143 |

9 WHISKERS-Mediated Transformation of Maize

J.F. PETOLINO, M. WELTER, and C. QIHUA CAI

| | | |
|-----|---|-----|
| 9.1 | Introduction | 147 |
| 9.2 | Preparation of Purified DNA Fragments | 147 |
| 9.3 | Establishment and Maintenance of Embryogenic Suspension Cultures | 150 |
| 9.4 | DNA Delivery via WHISKERS | 152 |
| 9.5 | Transgene Copy Number Estimation | 153 |
| 9.6 | Regeneration of Transgenic Plants and Progeny | 157 |

| | |
|---|-----|
| 9.7 Conclusions and Future Perspectives | 157 |
| References | 158 |

10 Genetic Transformation of Soybean with Biolistics

D. SIMMONDS

| | |
|---|-----|
| 10.1 Introduction | 159 |
| 10.2 Tissue Culture and Plant Regeneration | 160 |
| 10.2.1 Genotype Specificity | 160 |
| 10.2.2 Initiation and Repetitive Proliferation of Somatic Embryogenic Cultures | 161 |
| 10.2.3 Embryo Histodifferentiation and Maturation | 163 |
| 10.2.4 Germination, Conversion and Plant Fertility | 163 |
| 10.3 Transformation | 164 |
| 10.3.1 Gene Delivery | 164 |
| 10.3.2 Target Tissue Optimization | 165 |
| 10.3.3 Selection | 165 |
| 10.3.4 Transgenic Plant Recovery | 166 |
| 10.4 Conclusions | 167 |
| 10.5 Protocol | 168 |
| 10.5.1 Induction and Maintenance of Proliferative Embryogenic Cultures | 168 |
| 10.5.2 Transformation | 168 |
| 10.5.3 Selection | 169 |
| 10.5.4 Plant Regeneration | 169 |
| References | 170 |

11 Genotoxic Effects of Tungsten Microparticles Under Conditions of Biolistic Transformation

J. BUCHOWICZ and C. KRYSIAK

| | |
|--|-----|
| 11.1 Introduction | 175 |
| 11.2 Biological Significance of Tungsten | 175 |
| 11.2.1 Early Observations on Biological Effects of Tungsten | 175 |
| 11.2.2 Catalytic Activity of Simple Tungsten Compounds | 176 |
| 11.2.3 Tungstoenzymes | 176 |
| 11.2.4 Tungsten-DNA Interaction | 177 |
| 11.3 Tungsten Microparticles in Biotechnological Applications | 178 |
| 11.3.1 Biolistic Transformation | 178 |
| 11.3.1.1 An Overview | 178 |
| 11.3.1.2 Technical Details | 180 |
| 11.3.2 Biolistic Inoculation and Related Applications of Tungsten Particles | 181 |
| 11.4 Assessment of Tungsten-Induced DNA Lesions | 182 |
| 11.4.1 Electrophoretic Analysis of Tungsten-Damaged Plasmid DNA | 182 |

| | |
|---|------------|
| Contents | XV |
| 11.4.2 A Modified TUNEL Method for Detection of Cellular DNA Fragmentation | 184 |
| 11.5 Post-Bombardment Inhibition of Somatic Embryogenesis | 186 |
| 11.6 Concluding Remarks | 188 |
| References | 188 |
| Subject Index | 195 |

List of Contributors

T. ARONEN

Finnish Forest Research Institute, Punkaharju Research Station,
Finlandiantie 18, 58450 Punkaharju, Finland

P.C. BINSFELD

Center of Biotechnology, Federal University of Pelotas – UFPel, Brazil, Campus
Universitário, Caixa Postal 354, 96010-900, Pelotas-RS, Brazil

J. BUCHOWICZS

Institute of Biochemistry and Biophysics, Polish Academy of Sciences,
5A Pawinskiego Street, 02-106 Warsaw, Poland

D.H. CLAPHAM

Department of Plant Biology and Forest Genetics, Swedish University of
Agricultural Sciences, Box 7080, 750 07 Uppsala, Sweden

G. DEN HERDER

Department of Plant Systems Biology, Flanders Interuniversity Institute
for Biotechnology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent,
Belgium

C.C. EADY

New Zealand Institute for Crop & Food Research Limited, Private Bag 4704,
Christchurch, New Zealand

M. ELFSTRAND

Department of Plant Biology and Forest Genetics, Swedish University of
Agricultural Sciences, Box 7080, 750 07 Uppsala, Sweden

N. GÖZÜKIRMIZI

TUBITAK, Research Institute for Genetic Engineering and Biotechnology
2141470 Gebze, Kocaeli, Turkey

E.R. GOLD

Bell Chair in e-Governance, Faculty of Law, McGill University, 3664 Peel Street,
Montreal, H3A 1W9, Canada

S. GOORMACHTIG

Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

F. GÜREL

Istanbul University, Department of Molecular Biology and Genetics 34459 Vezneciler, Istanbul, Turkey

H. HÄGGMAN

Finnish Forest Research Institute, Punkaharju Research Station, Finlandiantie 18, 58450 Punkaharju, Finland and Department of Biology, University of Oulu, P. O. Box 3000, 90014 Oulu, Finland

M. HOLSTERS

Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

M. KARIMI

Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

C. KRYSIAK

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 5A Pawinskiego Street, 02-106 Warsaw, Poland

J.A. MCCOMB

Biological Sciences, Murdoch University, Murdoch, W.A. 6150, Australia

J.F. PETOLINO

Dow AgroSciences, 9330 Zionsville Road, Indianapolis, Indiana 46268, USA

C. QIHUA CAI

Dow AgroSciences, 9330 Zionsville Road, Indianapolis, Indiana 46268, USA

D. SIMMONDS

Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, C.E.F. Building 21, Ottawa, Ontario K1A 0C6, Canada

D. TJOKROKUSUMO

Biological Sciences, Murdoch University, Murdoch, W.A. 6150, Australia

D. TOMES

7300 NW 62nd Avenue, P.O. 1004, Johnston, Iowa 50131, USA

W. VAN DE VELDE

Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

M. VAN MONTAGU

Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

S. VON ARNOLD

Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Box 7080, 750 07 Uppsala, Sweden

M. WELTER

Dow AgroSciences, 9330 Zionsville Road, Indianapolis, Indiana 46268, USA

S.J. WYLIE

Biological Sciences, Murdoch University, Murdoch, W.A. 6150, Australia

Z. ZHAO

7300 NW 62nd Avenue, P.O. 1004, Johnston, Iowa 50131, USA