Soil Biology

Series Editor: Ajit Varma

4

Stéphane Declerck
Désiré-Georges Strullu
J.-André Fortin (Eds.)

In Vitro Culture of Mycorrhizas

With 84 Figures, 13 in Color

Springer

Prof. Dr. Stéphane Declerck
Université catholique de Louvain (UCL)
Mycothèque de l'Université catholique
de Louvain (MUCL)
Unité de microbiologie
3 Place Croix du Sud
1348 Louvain-la-Neuve
Belgium
e-mail: declerck@mbla.ucl.ac.be

Prof. Dr. Désiré-Georges Strullu
Université d'Angers
Laboratoire de Phytonique
2 Bd. Lavoisier
49045 Angers Cedex
France
e-mail: strullu@univ-angers.fr

Prof. J. André Fortin
Université Laval
Département de Sciences du Bois
et de la Forêt
Centre de Recherche en Biologie Forestière
Sainte-Foy, Québec G1K 7P4
Canada
e-mail: j.andre.fortin@videotron.ca

Library of Congress Control Number: 2004116751

ISSN 1613-3382
ISBN-10 3-540-24027-6 Springer Berlin Heidelberg New York
ISBN-13 978-3-540-24027-3 Springer Berlin Heidelberg New York

Springer is a part of Springer Science + Business Media
springeronline.com

Cover design: *design&production*, Heidelberg, Germany
Typesetting and production: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig, Germany
31/3150-YL - 5 4 3 2 1 0 - Printed on acid-free paper

Foreword

The first 30 cm of the earth's surface represents a fragile and valuable ecosystem, thanks to which terrestrial plants, and indirectly animals and humans, can live. The microbial activity occurring in soil is largely responsible for its physical and nutritional quality. Among the micro-organisms living in soil, the arbuscular mycorrhizal (AM) fungi play a major role. They are present in all types of soil, everywhere on the planet, living in symbiotic association with the roots of most plant species. They have co-evolved with plants for 400 million years, improving their nutrition and resistance to various types of stress. Present practices in conventional agriculture, which introduce great amounts of chemicals, have eliminated or underexploited the AM symbiosis. The rational exploitation of AM fungi in sustainable agriculture, to help minimize the use of chemical fertilizers and pesticides, has been hampered by several biological characteristics of these micro-organisms: they cannot be grown in the absence of a plant host and their genetic structure is very complex.

Despite these limitations, biologists have made important progress in understanding better the functioning of AM fungi. An in vitro technique has been developed using mycorrhizal root organ cultures, which made it possible to investigate the genetics, cell biology and physiology of AM fungi. We can now be objective enough to critically evaluate the impacts the in vitro technique has had to improve our knowledge on mycorrhizal symbiosis. Moreover, more experiences in using the technique allows us to appreciate its limits, as well as its yet unexploited scientific potential. A review on the subject has been recently published by Fortin et al (2002).

Along the same lines, but in a much more comprehensive way, this book, through contributions from experienced specialists in the field, offers valuable insights into the most recent uses of the technique. It illustrates how important questions regarding germplasm collection, taxonomy, physiology and metabolism of arbuscular mycorrhizal fungi can be cleverly addressed by taking advantage of the in vitro system. It also reports how the technique has been extended to the culture of other symbiotic fungi. In a unique way, a root/fungus symbiosis normally occurring in soil is made accessible for various investigations: e.g. non-destructive microscope observations, reliable cell physiology studies, clean biochemical and molecular analyses, and highly controlled interaction studies with other micro-organisms. Be-

cause the system provides a way to cultivate in vitro an obligate biotrophic micro-organism, it can even be used to produce aseptically, for the first time, AM fungal inocula on an industrial scale.

Young scientists interested in mycorrhizal symbiosis will find in this book, not only valuable technical information, but also a rich source of inspiration for their research and for the further exploitation of the potential of mycorrhizal in vitro cultures. Like microscopy for cell biology, and the polymerase chain reaction for molecular biology, the mycorrhizal root organ culture system can be considered a critical step in the scientific history of mycorrhiza R&D. This book will certainly provide convincing evidence to support this assertion.

Guillaume Bécard

Acknowledgements

We would like to thank the authors for their contributions and for all their efforts to produce and up-date a literature overview of so many different topics. The success of this book lies in their remarkable contributions. We owe special thanks to Professor Ajit Varma, who invited us to edit this book in the Series, *Soil Biology*. His constant encouragement is highly appreciated. We address special thanks to Dr. Jutta Lindenborn for her patience, dedicated editorial assistance and professionalism during all steps of this book production and to Dr. Dieter Czeschlik, who found our research a valuable topic for this Springer series.

S.D. gratefully acknowledge the financial support from the Belgian Federal Science Policy Office (contract BCCM C3/10/003).

S. Declerck, D.G. Strullu, J.A. Fortin
November 2004

Contents

Part I State of the Art

Part II Systematics

Part V Root Organ Culture of Other Fungal Symbioses

Part VII Methodology

Contributors

Alok Adholeya
Centre for Mycorrhizal Research, Biotechnology and Management of Bioresources Division The Energy and Resources Institute (TERI), DS Block, India Habitat Centre, Lodhi Road, 110003 New Delhi, India

Bert Bago
Estación Experimental del Zaidín (CSIC), Dpto. Microbiología del Suelo y Sistemas Simbióticos, calle Profesor Albareda 1, 18008 Granada, Spain

Céline Bivort
Université catholique de Louvain (UCL), Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Custodia Cano
Estación Experimental del Zaidín (CSIC), Dpto. Microbiología del Suelo y Sistemas Simbióticos, calle Profesor Albareda 1, 18008 Granada, Spain

Andrew P. Coughlan
Université Laval, Centre de Recherche en Biologie Forestière, Pavillon C.-E.-Marchand, Sainte-Foy, Québec G1K 7P4, Canada

Sylvie Cranenbrouck
Université catholique de Louvain (UCL), Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Yolande Dalpé
Agriculture and Agri-Food Canada, Environment Health, 960 Carling Avenue, Ottawa K1A 0C6, Canada

Stéphane Declerck
Université catholique de Louvain (UCL), Mycothèque de l'Université catholique de Louvain (MUCL), Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Ivan Enrique de la Providencia
Université catholique de Louvain (UCL), Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Bruno Delvaux
Université catholique de Louvain (UCL), Unité des Sciences du Sol, 2/10 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Yves Desjardin
Université Laval, Centre de recherche en horticulture, FSAA, Québec G1K 7P4, Canada

Francisco Adriano de Souza
Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agrobiologia, BR 465-RJ, km 7, Caixa Postal 74505, CEP 23851-000 Seropédica, RJ, Brazil.

David D. Douds Jr.
US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA 19038, USA

Hervé Dupré de Boulois
Université catholique de Louvain (UCL), Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Roger Durand
Université du Littoral côte d'Opale, Laboratoire Mycologie/Phytopathologie/Environnement, 17 avenue BLERIOT, 62228 Calais, France

Annemie Elsen
Katholieke Universiteit Leuven, Laboratory of Tropical Crop Improvement, Kasteelpark Arenberg 13, 3001 Leuven, Belgium

Joël Fontaine
Université du Littoral côte d'Opale, Laboratoire Mycologie/Phytopathologie/Environnement, 17 avenue BLERIOT, 62228 Calais, France

J.-André Fortin
Université Laval, Département de Sciences du Bois et de la Forêt, Centre de Recherche en Biologie Forestière, Sainte-Foy, Québec G1K 7P4, Canada

Amar Prakash Garg
Ch. Charan Singh University, Department of Microbiology, Meerut, Uttar Pradesh, India

Giovanna Maria Giomaro
Università degli Studi di Urbino "Carlo BO", Istituto di Botanica & Orto Botanico "Pierina Scaramella", Via Bramante 28, 61029 Urbino, Italy

Anne Grandmougin-Ferjani
Université du Littoral côte d'Opale, Laboratoire Mycologie/Phytopathologie/Environnement, 17 avenue BLERIOT, 62228 Calais, France

Solveig Hehl
Carl Zeiss Jena GmbH, Advanced Imaging Microscopy Division, Carl-Zeiss Promenade 10, 07745 Jena, Germany

Cinta Hernandez-Sebastia
University of North Carolina at Chapel Hill, Dept. of Biology, Coker Hall CB# 3280, Chapel Hill, NC-27701, USA

Erik Joner
Agricultural University of Norway, Department of Biotechnological Sciences, N-1432 Aas, Norway

Nathalie Kruyts
Université catholique de Louvain (UCL), Unité des Sciences du Sol, 2/10 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Rina Kumari
Jawaharlal Nehru University, School of Life science, 110067 New Delhi and Ch. Charan Singh University, Department of Microbiology, Meerut, Uttar Pradesh, India

Gerald Nagahashi
US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, Pennsylvania 19038, USA

Ralf Oelmüller
University of Jena, Institute für Allgemeine Botanik, Dornburger Strasse 159, Jena, Germany

Tatjana Peskan
University of Jena, Institute für Allgemeine Botanik, Dornburger Strasse 159, Jena, Germany

Huong Giang Pham
Jawaharlal Nehru University, School of Life science, 110067 New Delhi, India

Yves Piché
Université Laval, Centre de Recherche en Biologie Forestière, Pavillon C.-E.-Marchand, Sainte-Foy, Québec G1K 7P4, Canada

Ram Prasad
Jawaharlal Nehru University, School of Life science, 110067 New Delhi and Ch. Charan Singh University, Department of Microbiology, Meerut, Uttar Pradesh, India

Laurent Renard
Université catholique de Louvain (UCL), Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Gervais Rufyikiri
Belgian Nuclear Research Centre (SCK*CEN, Foundation of Public Utility), Radiation Protection Research Department, Radioecology Section, Boeretang 200, 2400 Mol, Belgium.

Minu Sachdev
Jawaharlal Nehru University, School of Life-Sciences, 110067 New Delhi, India

Arthur Schüßler
Darmstadt University of Technology (TUD), Department of Biology, Institute of Botany, Schnittspahnstrasse 10, 64287 Darmstadt, Germany

Sylvie Séguin
Agriculture and Agri-Food Canada, Environment Health, 960 Carling Avenue, Ottawa K1A 0C6, Canada

Nathalie Séjalon-Delmas
UMR 5546, Equipe de Mycologie Végétale, Pole de Biotechnologie Végétales, Chemin de Borde-Rouge, B.P. 42617, 31 326 Castanet-Tolosan, France

Irena Sherameti
Univesity of Jena, Institute für Allgemeine Botanik, Dornburger Strasse 159, Jena, Germany

Anjana Singh
Tribhuvan University, Department of Microbiology, Kathmandu, Nepal

Reena Singh
Centre for Mycorrhizal Research, Biotechnology and Management of Bioresources Division The Energy and Resources Institute (TERI), DS Block, India Habitat Centre, Lodhi Road, 110003 New Delhi, India

Davide Sisti
Università degli Studi di Urbino "Carlo BO", Istituto di Botanica & Orto Botanico "Pierina Scaramella", Via Bramante 28, 61029 Urbino, Italy

Marc St-Arnaud
Institut de Recherche en Biologie Végétale, Jardin Botanique de Montréal, 4101 est, Montréal, Québec HIX 2B2, Canada

Désiré-Georges Strullu
Université d'Angers, Laboratoire de Phytonique, 2 Bd. Lavoisier, 49045 Angers Cedex, France

Yves Thiry
Belgian Nuclear Research Centre (SCK*CEN, Foundation of Public Utility), Radiation Protection Research Department, Radioecology Section, Boeretang 200, 2400 Mol, Belgium.

Pragati Tiwari
Centre for Mycorrhizal Research, Biotechnology and Management of Bioresources Division The Energy and Resources Institute (TERI), DS Block, India Habitat Centre, Lodhi Road, 110003 New Delhi, India

Ajit Varma
Jawaharlal Nehru University, School of Life science, New Delhi 110067, India. *Current Address:* Amity Institute of Herbal and Microbial Research, Noida, India

Horst Vierheilig
Universität für Bodenkultur Wien (BOKU), Institut für Pflanzenschutz, Peter-Jordan-Strasse 82, 1190 Wien, Austria

Liesbeth Voets
Université catholique de Louvain (UCL), Unité de microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Elke Wolf
Darmstadt University of Technology (TUD), Department of Biology, Institute of Botany, Schnittspahnstrasse 10, 64287 Darmstadt, Germany

Vikas Yadav
Jawaharlal Nehru University, School of Life-Sciences, 110067 New Delhi, India

Alessandra Zambonelli
Università degli Studi di Bologna, Dipartimento di Protezione e Valorizzazione Agroalimentare, Via Fanin 46, 40127 Bologna, Italy

Part I
State of the Art

1 In Vitro Culture of Mycorrhizas

J. André Fortin[1], Stéphane Declerck[2], Désiré-Georges Strullu[3]

1 Introduction

Symbiosis with fungi has been determinant for the evolution of vascular plants since their apparition on land. Devonian Rhynia fossils (400×10^6 years old) permit one to observe, in the lower part of their stems, fungal structures closely resembling modern Glomales (Pirozynsky and Malloch 1975). Molecular clocks also permitted one to date the early evolution of Glomales back to about 400×10^6 years (Simon et al. 1993). It seems that associations with some soil fungi were a prerequisite for the evolution of autotrophic land plants, as was also the case with lichens. Plant fossils from several geological periods show the presence of mycorrhizal structures.

During this evolution, arbuscular mycorrhizal (AM) fungi became totally dependent on their host, i.e. obligate symbionts. Today, at our present state of knowledge, it is impossible to grow these fungi independently from a host plant. This also explains why the understanding of the significance of AM fungi in the life of vascular plants and ecosystem dynamics came so late in the second part of the 20th century.

The obligate nature of the AM fungi has always, and still is making it difficult to study most aspects of the biology of these ubiquitous and fundamentally important fungi, including their functioning and roles in terrestrial ecosystems.

Since the mid-1980s, the use of root-organ culture has opened new vistas on several aspects of the AM symbiosis (Fortin et al. 2002). This review gives an idea of the work accomplished but, above all, what remains to be achieved. We feel that this contribution will also encourage more scientists

[1]Université Laval, Faculté de Foresterie et Géomatique, Centre de Recherche en Biologie Forestière, Ste-Foy, QC G2A 1M2, Canada, Tel.: +1-418-6562131, ext. 6119, E-mail: j.andre.fortin@videotron.ca
[2]Université Catholique de Louvain, Mycothèque de l'Université Catholique de Louvain (MUCL), Unité de Microbiologie, 3 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium
[3]Laboratoire de Phytonique, Université d'Angers, 2 Bd. Lavoisier, 49045 Angers cedex, France

Soil Biology, Volume 4
In Vitro Culture of Mycorrhizas
(ed. by S. Declerck, D.-G. Strullu, and A. Fortin)

to use this approach for increasing innovative research. It also convinced us that there was a need for a more extensive document reporting precise methodologies, disseminating more thoroughly the new knowledge being gleaned, elucidating the potential for diversified use of the method, and also identifying new avenues for further research.

It has become obvious that all areas of AM fungi biology per se, as well as the biology of the symbiotic relationship, have been revisited using monoxenic cultures. Cultivation of AM fungi on root cultures has shed new light on their molecular biology, cytology, genetics, physiology, systematics and phylogeny, which has since received a tremendous innovative momentum. Large-scale industrial production of biologically clean AM inocula produced on root cultures has also become a reality in some countries, including India and Canada.

This first chapter aims to summarize some of the principal findings extensively discussed in the chapters of this book. Several terms related to the so-called in vitro culture of AM fungi have been used in the literature to designate one and the same concept (in vitro, monoxenic, monoaxenic root-organ culture and root culture). For clarity and uniformity throughout this volume, we propose the following standard definitions. A monoxenic culture of an arbuscular mycorrhizal (AM) fungus is a reproducible and contaminant-free, in vitro co-culture between a root organ and a glomalean species. This co-culture should be regarded as continuous if "*the endophyte is maintained* in vitro *indefinitely. It must be subcultured in order to maintain and increase its biomass*" (Bécard and Piché 1992). A root-organ culture is the indefinite culture on a synthetic medium of a transformed or non-transformed, excised root.

The interest of aseptically grown root organs to cultivate AM fungi was communicated to other organisms, namely ectomycorrhizal fungi, where a large number of species can be grown without a host but where several entities see their development improved (e.g. Tuberales). We took the opportunity in this book to underline the interest on basidiomycetes belonging to Sebacinae. Although easily cultivated axenically, these fungi mimic the effects of quite a number of AM fungi on plant growth.

2
A Tool for Germplasm Collection

For the study of micro-organisms, researchers, regardless of their field of interest, must have access to reliable sources of aseptic, properly identified and properly conserved germplasm banks. Such banks must have the recognition of the World Federation of Culture Collection (WFCC). Pot culture-based banks such as BEG and INVAM are most useful and will

remain so, until successful monoxenic cultivation of most existing Glomeromycota is achieved. In the light of Chapter 2, presented by Declerck, Séguin and Dalpé, this is not likely to happen very rapidly, since the number of cultivated species is less than 10% of the estimated 180 species existing in the world. However, increasing numbers of species belonging to most genera are documented in the literature, and are expected to rapidly become available to the scientific community.

3
A Tool for Systematics and Biodiversity

AM fungal taxonomists represent a rare breed, and the support they receive rarely compares to the importance of the issues. Yet, new approaches will have to be developed if successful cultivation of more diversified species of AM fungi is to be achieved. Observations of AM fungi behaviour in bi-compartments suggest that, paradoxically, the mycorrhizal root vicinity is not favourable for the development of extra-matrical mycelium or spore production (Fig. 2 in Fortin et al. 2002). This suggests that bi-compartments should be more widely used in an attempt to cultivate recalcitrant species. Not all soil microbes can grow in completely synthetic medium, thus the presence of soil extracts is often a key to successful cultivation. It should not be assumed that the relationship with the host plant fulfils all the nutriment requirements of AM fungi. Genetic derivation of subcultured AM fungi is often evoked and usually assumed. There is a need for rigorous research regarding this question, along with the development of methods for long-term conservation. In biology, every scientific activity must be based on precise knowledge of the systematic position of the organisms being studied; reliable nomenclature is a prerequisite for organizing knowledge in a useful manner, and assuring continuity and reproducibility of results. AM fungi taxonomists are dealing with fungi living in the soil, a complex environment showing a minimum of morphological characters. Above all, these organisms cannot be cultivated in the absence of a host plant. In Chapter 3, Dalpé, Cranenbrouck, Séguin and Declerck present the problematics of AM fungi systematics, demonstrating the usefulness of monoxenic culture for precise morphological, biochemical and molecular observations of the different steps of their lifecycle. Obviously, monoxenic cultures of AM fungi play a key role in improving our knowledge of their taxonomic classification, their biodiversity and their functionality, in natural as well as managed ecosystems of the world. More graduate students should be encouraged to make a career in AM fungal taxonomy, adding molecular tools to classical approaches.

4
Life Cycle of *Glomus* spp.

The most abundant, at least in managed ecosystems, and the most easily captured, isolated and maintained AM fungi on root cultures belong to the genus *Glomus*. Cultivation of several species and strains has permitted us to trace the life cycle of these species. Dalpé, de Souza and Declerck present (Chap. 4) a detailed step-by-step description of a typical *Glomus*, putting together virtually all the research published up to now on morphological, structural and biochemical aspects of their biology. They also present specific conditions necessary for promoting the development of given stage of the life cycle, i.e. spore germination. Since several *Glomus* spp. can be obtained, observed and maintained on root explants, this should encourage some scientists to cultivate an ever-increasing number of *Glomus* species; the framework recommended in Chapter 2, on the maintenance of AM fungal germplasms, should be strictly followed.

5
Life History of Gigasporaceae

Glomus spp. are rather easy to cultivate monoxenically, but this is not the case with the majority of other AM fungal genera. de Souza, Dalpé, Declerck, de la Providencia and Séjalon-Delmas put together their experience with Gigasporaceae and present an overview of their life cycle (Chap. 5). The fact that most information is based on non-aseptic systems illustrates the challenge that these AM fungi present for their continuous monoxenic cultivation. One of the difficulties is that they often require a longer cultivation period (several months) to produce their first spores on root organs, as compared to only 10 weeks in pot culture. The authors of this chapter mention that spore production comes after senescence of the root. We suggest that selectively weakening or killing (physically or chemically) the root might possibly trigger spore production.

6
Effects of Environmental Factors on Hyphal Growth and Branching

AM fungi must find a compatible host plant to complete their life cycle. In Chapter 6, Nagahashi and Douds present the environmental factors, including light, gaseous or volatile compounds and non-volatile chemical compounds, which affect pre-symbiotic hyphal branching and growth.

Purified chemicals such as some flavonols can stimulate the growth of AM fungi. These authors review the germ tube responses to different interactions between: (1) a gaseous compound and chemicals, (2) different soluble chemical compounds and (3) chemical compounds and light. It appears that AM spores can generally germinate without the presence of root exudates, but the components of the exudates can stimulate fungal growth, hyphal branching and root colonization. It has been demonstrated that multiple genes are expressed when a germinated spore is treated with host root exudates. Recent evidence suggests that we should be aware that there might be different factors for elongation growth and hyphal branching. Not every environmental factor affects AM fungi positively. In addition to chemical components of exudates and volatile compounds, the authors demonstrated that a third physical factor, light, stimulates hyphal branching. In particular, blue light and root exudates appear to trigger the same second messenger involved in the hyphal branching response.

7
Questioning the Value of Monoxenic Cultures

In Chapter 7, Bago and Cano present an interesting discussion concerning seven main questions:

Are AM monoxenic cultures devices too artificial to trust? Does primary colonization by AM fungi occur in young roots? Do hyphae exit the root after symbiosis begins? Are branched absorbing structures (BAS) formed by all glomalean fungi or are artefacts formed under monoxenic conditions? Are there any differences in the development of AM fungi in monoxenic vs. soil cultures? Are AM monoxenic liquid cultures accurate enough to use? What else can monoxenic cultures offer regarding the study of AM fungal biology? In this chapter, the authors present an overview on subjects of high potential interest for those working with AM fungi, either for scientific or commercial purposes.

8
AM Fungi; Host and Non-Host

Arbuscular mycorrhizal fungi can be found in the roots of 80% of all vascular plant species. Generally, Brassicaceae are described as being non-mycorrhizal, but numerous conflicting papers report mycorrhizal associations in many taxa of the Brassicaceae (*Arabidopsis*, *Brassica*, *Cardamina*) and the Chenopodiaceae (sugar beet and spinach). Chemical factors may be involved in reducing the infection. The establishment of mycorrhizal

symbiosis involves a process leading to the recognition and compatibility between the two partners, but the mechanism governing these phenomena is not well understood. In Chapter 8, Vierheilig and Bago discuss the host and non-host impact on the physiology of the AM symbiosis. The authors identify several phases of the root–AM fungal interaction: (1) asymbiotic phase (axenic culture), when the fungus germinates and grows in the absence of plant signals, (2) pre-symbiotic phase, when the fungus germinates and grows in the presence of signal exudates, and (3) symbiotic phase, when the fungus has penetrated the root and formed intraradical arbuscules. The latter phase is difficult to obtain in monoxenic culture, and fewer physiological data are available. The effects of pH, temperature, CO_2 and light on spore germination and hyphal asymbiotic growth of AM fungi are presented first. In a second point concerning pre-symbiotic AM fungus growth, the data discussed show the importance of root exudates favourable to AM fungi for the successful establishment of the symbiosis. At least at the pre-symbiotic phase of the association, some AM non-host plants and myc$^-$ plants seem to share mechanisms affecting their susceptibility to AM fungi. The perception of AM fungi by the plant before root colonization is poorly documented. It has been recently hypothesised that a more favourable environment for root penetration is created by the host in the presence of fungal signals.

9
Carbon and Lipid Metabolism

Great possibilities are offered by monoxenic culture to study different aspects during the formation of the AM association. The knowledge of these interactions progresses at cellular, molecular and biochemical levels. It is generally accepted that up to 20% of the photosynthetically fixed carbon is transferred from the plant to the AM fungi. Intraradical hyphae incorporate plant-derived hexose, which is converted to typical storage forms, trehalose and glycogen, but extraradical mycelium is incapable of taking up sugars. A gene encoding for a transmembrane sugar transporter was cloned from mycorrhizal roots of *Medicago trunculata*. According to Harrison (1996), this transporter (*Mtst1*) was designed as a hexose transporter by activity measured in yeast. The failure of AM fungi to complete their life cycle in the absence of roots could originate from the control by the plant of fungal genes involved in carbon transport and metabolism. On this basis, Grandmougin-Ferjani, Fontaine and Durand (Chap. 9) present the monoxenic culture technique as a tool for the establishment of the lipid composition of AM fungi. Lipid droplets are abundant in spores and vesicles of AM fungi, and biochemical studies indicate that lipids can represent up

to 45% of the fungal dry biomass. The authors give a comparison between lipid analyses of AM fungi (*Glomus intraradices*) obtained by in vitro and in vivo systems. They also propose the use of monoxenic cultures as a tool for the evaluation of AM fungi in host root tissue. AM monoxenic cultures, combined with isotopic labelling techniques, enable a better understanding of lipid metabolism of AM fungi. Moreover, these authors note that the lipid metabolism of AM fungi is still unclear, since results from ^{14}C and ^{13}C labelling seem to be contradictory. RMN studies of lipids suggest that obligate biotrophy of AM fungi could be due to a lack of, or insufficient ability of neutral lipid biosynthesis in both germinating spores and extraradical mycelium. Cloning and expression analysis of genes encoding enzymes involved in lipid biosynthesis are now required. The use of AM monoxenic cultures has clarified some aspects of the symbiotic interactions. Moreover, there are certainly some differences in AM fungi development when grown in vitro (monoxenically) and in vivo, but these could be reduced.

10
Monoxenic Culture and Physiology of in Vitro Grown Plants

Desjardins, Piché and Sebastia (Chap. 10) illustrate how AM fungi produced on root cultures can be useful in the study of the comparative physiology of in vitro cultivated plants, especially in relation to water stress and sink–source relationships. The data demonstrate that the mycorrhizal inoculation of in vitro propagated plants is very promising in acquiring healthy plants, and improves the adaptation of such plants when transferred under natural conditions. In a different context, a review on this subject would be of great practical interest.

11
Nutrient Dynamics in AM Monoxenic Cultures

According to Rufyikiri, Kruyts, Declerck, Thiry, Delvaux, Dupré de Boulois and Joner (Chap. 11), the monoxenic culture system offers three major advantages for element transport studies: (1) bio-sorption and affinity studies at low concentration; (2) modification of the speciation of a defined element due only to its interactions with the AM fungus; and (3) determination of specific uptake and flux rates. Monoxenic culture systems are useful in studies involving essential elements (N and P) and radionuclides (U and Cs). AM fungi take up and translocate these elements. As AM fungi are an important part of the rhizospheric micro-organism biomass, the uptake of radionuclides by the extraradical mycelium has ecological significance –