

Ajit Varma · Ram Prasad
Narendra Tuteja *Editors*

Mycorrhiza - Eco-Physiology, Secondary Metabolites, Nanomaterials

Fourth Edition

 Springer

Mycorrhiza - Eco-Physiology, Secondary Metabolites, Nanomaterials

Ajit Varma • Ram Prasad • Narendra Tuteja
Editors

Mycorrhiza - Eco-Physiology, Secondary Metabolites, Nanomaterials

Fourth Edition

 Springer

Editors

Ajit Varma
Amity Institute of Microbial Technology
Amity University Uttar Pradesh
Noida, Uttar Pradesh
India

Ram Prasad
Amity Institute of Microbial Technology
Amity University Uttar Pradesh
Noida, Uttar Pradesh
India

Narendra Tuteja
Amity Institute of Microbial Technology
Amity University Uttar Pradesh
Noida, Uttar Pradesh
India

ISBN 978-3-319-57848-4

ISBN 978-3-319-57849-1 (eBook)

DOI 10.1007/978-3-319-57849-1

Library of Congress Control Number: 2017944110

© Springer International Publishing AG 2017

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, 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.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Springer imprint is published by Springer Nature

The registered company is Springer International Publishing AG

The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

The pressure on plant production systems is steadily increasing. At first, areas which could be used for the cultivation of plants are getting smaller because more and more space is used for other anthropogenic activities. Secondly, environmental constraints like soil erosion, salinization, or flooding lead to periodical yield losses and finally to the decision to give up a particular region for plant production. Thirdly, the use of pesticides becomes difficult, because the application of more and more compounds is not permitted anymore or they have lost their effectiveness. The development of new agents is time and cost intensive, and it is questionable if there will be enough of such new agents to substitute the compounds which are disappearing from the market. Under these circumstances, the application of plant-interacting microorganisms in plant production systems becomes more and more a realistic alternative and might be the only chance in the future to produce enough food for a growing world population. Among such microorganisms, mycorrhizal fungi fill a particular position. With their hyphae colonizing at the same time the root and the surrounding soil, they connect the inside and the outside of the plant. In this so-called mycorrhizosphere, they bring together all physical, chemical, and biological factors of the terrestrial environment with the physiology of the plant.

The book “Mycorrhiza: Eco-Physiology, Secondary Metabolites, Nanomaterials” gives an excellent overview of the current state of the art from basic to applied mycorrhizal research. It covers different types of interactions including those between the orchid mycorrhizal fungus *Piriformospora indica* and non-orchid plants. Several chapters describe more basic aspects but nevertheless important for application. Carbon flux in mycorrhizal plants has more and more to be the basis for predicting the outcome of mycorrhizal interactions. Functional diversity must be managed for an adapted application in the field. Also, plant–fungus signaling needs a better understanding. Most chapters, however, describe where and how mycorrhizal fungi can be used in plant production under difficult conditions and show in this way how broad the possibilities for application can be. I therefore congratulate the editors that they brought together so many different facets of basic and applied mycorrhizal

research. I also congratulate you on holding this book in your hand and ask you to read at least some of the highly interesting chapters.

Erfurt, Germany
20 March 2017

Philipp Franken

Preface

German pathologist A.B. Frank (1885) coined the term Mycorrhiza which literally means fungus roots. These fungi aid in the productivity of plants *via* the formation of dynamic associations with plant roots. Mycorrhiza is considered a fundamental part of the root colonization and stabilization of plants on terrestrial habitats. The symbiotic associations formed are an important subject to evaluate various opportunities using modern tools of biotechnology. The possibilities of genetically manipulating these associations have led to the optimization of plant productivity in ecosystems with minimal risk of environmental damage.

This volume of the mycorrhiza book gives exemplary insight into the advancements in mycorrhizal studies. This edition extensively illuminates the ecophysiological aspects, secondary metabolite production, and interaction of mycorrhiza with nanomaterials. The ability of mycorrhiza to provide resistance against various abiotic and biotic stresses has been explored in various parts of this edition. In addition to providing resistance, mycorrhizas are known to increase secondary metabolite production of plants. Therefore, various studies have been conducted to elucidate the mycorrhiza-induced increase of secondary metabolites in various economically important and medicinal plants. Interaction studies of nanomaterials with mycorrhiza have also been a subject of recent interest.

It is hoped that this new edition will interest readers in the latest outcomes of mycorrhiza research and also encourage young researchers to prove the challenging field of these studies.

This volume consists of 18 chapters covering the diverse mycorrhizal associations by 57 eminent academicians and subject specialists.

We are grateful to the many people who helped to bring this volume to light. We wish to thank Hanna Hensler-Fritton, Isabel Ullmann, and Man-Thi Tran Springer Heidelberg, for generous assistance and patience in finalizing the volume. Finally, special thanks go to our families, immediate, and extended, not forgetting those who have passed away, for their support or their incentives in putting everything together. Editors in particular are very thankful to Dr. Ashok K. Chauhan, Founder President of the Ritnand Balved Education Foundation (an umbrella organization of

Amity Institutions), New Delhi, for the kind support and constant encouragement received. Special thanks are due to my esteemed faculty colleagues and dear student Ms Diksha Bhola and other technical staff.

Amity University Uttar Pradesh
Noida, India

Ajit Varma
Ram Prasad
Narendra Tuteja

Contents

1	Carbon Fluxes in Mycorrhizal Plants	1
	Veronika Řezáčová, Tereza Konvalinková, and Jan Jansa	
2	Basic and Applied Research for Desert Truffle Cultivation	23
	Asunción Morte, Manuela Pérez-Gilabert, Almudena Gutiérrez, Francisco Arenas, José Eduardo Marqués-Gálvez, Juan Julián Bordallo, Antonio Rodríguez, Luis Miguel Berná, Cecilia Lozano-Carrillo, and Alfonso Navarro-Ródenas	
3	The Role of Arbuscular Mycorrhizal Fungi and the Mycorrhizal-Like Fungus <i>Piriformospora indica</i> in Biocontrol of Plant Parasitic Nematodes	43
	Ruchika Bajaj, Ram Prasad, Ajit Varma, and Kathryn E. Bushley	
4	Mycorrhizal Fungi Under Biotic and Abiotic Stress	57
	Manoj Kumar, Ram Prasad, Vivek Kumar, Narendra Tuteja, and Ajit Varma	
5	Role of Arbuscular Mycorrhizal Fungi (AMF) in Salinity Tolerance and Growth Response in Plants Under Salt Stress Conditions	71
	Mahesh Borde, Mayura Dudhane, and Mohan Kulkarni	
6	Arbuscular Mycorrhizal Technology Based on Ecosystem Services Rendered by Native Flora for Improving Phosphorus Nutrition of Upland Rice: Status and Prospect	87
	Dipankar Maiti, Neha Nancy Toppo, Mukesh Nitin, and Binit Kumar	
7	Arbuscular Mycorrhizal Fungi in Redeeming Arsenic Toxicity in Plants	107
	Surbhi Sharma, Neeraja Singh, and Rupam Kapoor	
8	Co-cultivation of <i>Piriformospora indica</i> with <i>Azotobacter</i> sp.	135
	Prasun Bandyopadhyay and Ajit Varma	

9	Arbuscular Mycorrhizal Symbiosis: Genetic and Functional Diversity	149
	Rekha Pandey and Neera Garg	
10	Mycorrhizal Symbiosis: Ways Underlying Plant–Fungus Interactions	183
	Shaily Javeria, Vivek Kumar, Pratibha Sharma, Lakshman Prasad, Manoj Kumar, and Ajit Varma	
11	The Management of the Mycorrhizal Soil Infectivity: Ecological and Technical Approaches	209
	Adrien Lies, Yves Prin, Robin Duponnois, and Hicham Ferhout	
12	Reactive Oxygen Species (ROS) Metabolism and Signaling in Plant-Mycorrhizal Association Under Biotic and Abiotic Stress Conditions	223
	Manoj Nath, Deepesh Bhatt, Ram Prasad, and Narendra Tuteja	
13	Stimulated Growth of <i>Lycopersicum esculentum</i> CLA 1131 in Presence of <i>Piriformospora indica</i> and Vermicompost	233
	Reshma Tuladhar, Kenneth Shahi, Sujen Man Shrestha, Anjana Singh, and Ajit Varma	
14	Promotion and Value Addition to Some Important Medicinal Plants Under Saline Condition by Intervention of a Novel Mycorrhizal Formulation	247
	Priyanka Sharma, Hemesh Joshi, Amit C. Kharkwal, Narendra Tuteja, and Ajit Varma	
15	Cocultivation of <i>Piriformospora indica</i> and <i>Azotobacter chroococcum</i> for Production of Artemisinin	273
	Prasun Bandyopadhyay, Monika Arora, M.Z. Abdin, and Ajit Varma	
16	Microbial Symbiosis and Bioactive Ingredients of Medicinal Plants	283
	Divya Kilam, Priyanka Sharma, Abha Agnihotri, Amit Kharkwal, and Ajit Varma	
17	Cultivation of <i>Piriformospora indica</i> with Nanomaterial in Bioreactor	303
	Uma and Ajit Varma	
18	Understanding the Mycorrhiza-Nanoparticles Interaction	311
	Avinash Ingle, Dnyaneshwar Rathod, Ajit Varma, and Mahendra Rai	
	Index	325

List of Contributors

M.Z. Abdin Department of Biotechnology, Jamia Hamdard University, New Delhi, India

Abha Agnihotri Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Francisco Arenas Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Monika Arora Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Ruchika Bajaj Department of Plant Biology, University of Minnesota, St. Paul, MN, USA

Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Prasun Bandyopadhyay Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Luis Miguel Berná Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Deepesh Bhatt Department of Biotechnology, Shree Ramkrishna Institute of Computer Education and Applied Sciences, Affiliated to Veer Narmad South Gujarat University, Surat, Gujarat, India

Juan Julián Bordallo Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Mahesh Borde Department of Botany, Savitribai Phule Pune University, Pune, Maharashtra, India

Kathryn E. Bushley Department of Plant Biology, University of Minnesota, St. Paul, MN, USA

Mayura Dudhane Department of Botany, Savitribai Phule Pune University, Pune, Maharashtra, India

Robin Duponnois CIRAD, UMR LSTM, Montpellier, France

Hicham Ferhout AGRO NUTRITION, Carbonne, France

Neera Garg Department of Botany, Panjab University, Chandigarh, India

Almudena Gutiérrez Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Avinash Ingle Nanobiotechnology Laboratory, Department of Biotechnology, SGB Amravati University, Amravati, Maharashtra, India

Jan Jansa Laboratory of Fungal Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská, Czech Republic

Shaily Javeria Biological Control Laboratory, Division of Plant Pathology, IARI, New Delhi, India

Hemesh Joshi Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Rupam Kapoor Department of Botany, University of Delhi, Delhi, India

Amit C. Kharkwal Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Divya Kilam Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Tereza Konvalinková Laboratory of Fungal Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská, Czech Republic

Mohan Kulkarni Division of Biochemistry, Department of Chemistry, Savitribai Phule Pune University, Pune, India

Binit Kumar Central Rainfed Upland Rice Research Station (ICAR – National Rice Research Institute), Hazaribag, Jharkhand, India

Manoj Kumar Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Vivek Kumar Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Adrien Lies AGRO NUTRITION, Carbonne, France

IRD, UMR LSTM, Montpellier, France

Cecilia Lozano-Carrillo Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Dipankar Maiti Central Rainfed Upland Rice Research Station (ICAR – National Rice Research Institute), Hazaribag, Jharkhand, India

José Eduardo Marqués-Gálvez Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Asunción Morte Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Manoj Nath Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Alfonso Navarro-Ródenas Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Mukesh Nitin Central Rainfed Upland Rice Research Station (ICAR – National Rice Research Institute), Hazaribag, Jharkhand, India

Rekha Pandey Department of Botany, Panjab University, Chandigarh, India

Manuela Pérez-Gilabert Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Campus de Espinardo, Murcia, Spain

Lakshman Prasad Biological Control Laboratory, Division of Plant Pathology, IARI, New Delhi, India

Ram Prasad Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Yves Prin IRD, UMR LSTM, Montpellier, France

Mahendra Rai Nanobiotechnology Laboratory, Department of Biotechnology, SGB Amravati University, Amravati, Maharashtra, India

Dnyaneshwar Rathod Nanobiotechnology Laboratory, Department of Biotechnology, SGB Amravati University, Amravati, Maharashtra, India

Veronika Řezáčová Laboratory of Fungal Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská, Czech Republic

Antonio Rodríguez Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Murcia, Spain

Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Kenneth Shahi Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Pratibha Sharma Biological Control Laboratory, Division of Plant Pathology, IARI, New Delhi, India

Priyanka Sharma Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Surbhi Sharma Department of Botany, University of Delhi, Delhi, India

Sujen Man Shrestha Nepal Academy of Science and Technology, Lalitpur, Nepal

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

Neeraja Singh Department of Botany, University of Delhi, Delhi, India

Neha Nancy Toppo Central Rainfed Upland Rice Research Station, ICAR-National Rice Research Institute (formerly Central Rice Research Institute), Hazaribag, Jharkhand, India

Reshma Tuladhar Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Narendra Tuteja Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Uma Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Ajit Varma Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India

Chapter 1

Carbon Fluxes in Mycorrhizal Plants

Veronika Řezáčová, Tereza Konvalinková, and Jan Jansa

Abstract Although declared as a research priority more than 40 years ago, the knowledge about the magnitude and mechanisms of carbon (C) fluxes between plants and their mycorrhizal fungal symbionts remains fragmentary. In spite of a number of experiments with isotopically labeled C documented rapid and directed C transfer from the host plant to its mycobionts, the molecular mechanisms and their regulation involved in such a transport remain largely unknown. It seems that in many arbuscular mycorrhizal (AM) symbioses, the C costs remains well below 10% of the C fixed photosynthetically by the host plants. Higher values were detected in the past only under specific situations such as in young plants, under low light intensities, and/or for particular partner combinations, involving very costly (in terms of C demand) and little nutritionally beneficial AM fungi such as *Gigaspora* sp. Ecological context of the common mycorrhizal networks in terms of redistribution of symbiotic C costs and nutritional benefits on one hand and C movement through soil food webs beyond mycorrhizal hyphae on the other are briefly discussed in this chapter, and further research challenges and open knowledge gaps with respect to C fluxes in mycorrhizal plants are outlined.

1.1 Introduction

Mycorrhiza is one of the most common inter-species interactions on Earth, involving great majority (>90%) of plant species (Smith and Read 2008) and several groups (and functional guilds) of soil fungi (Nguyen et al. 2016; Prasad et al. 2017). This interaction involves bidirectional flows of matter between the symbiotic partners, exchanging mineral nutrients such as nitrogen (N) and phosphorus (P) for the reduced carbon (C) originating from plant photosynthesis (Ferrol et al. 2002). Several different types of the mycorrhizal symbiosis evolved during the history, involving different (often disjunctive) groups of symbiotic partners at both plant and fungal sides (Cairney 2000). Yet, the main function (nutrient for C

V. Řezáčová • T. Konvalinková • J. Jansa (✉)
Laboratory of Fungal Biology, Institute of Microbiology, Czech Academy of Sciences,
Václavská 1083, 14220 Prague, Czech Republic
e-mail: jansa@biomed.cas.cz

trading) is stunningly uniform across the different mycorrhizal types, with some remarkable deviations from this general pattern such as plant-bound C fluxing in orchid protocorms or mycoheterotrophic plants (Leake and Cameron 2010; Bever 2015).

Most efforts in mycorrhizal research have so far been dedicated to uncovering principles and diversity in nutritional and/or growth benefits the symbiosis confers to the plants or how the diversity of taxa and functions in the fungal communities affects the productivity/stability/diversity of the plant communities and vice versa (van der Heijden et al. 1998; Johnson et al. 2004; Munkvold et al. 2004; Cavagnaro et al. 2005). Less efforts have been dedicated to the role of mycorrhizas in sustainable soil use and in establishing and maintaining soil physical properties (e.g., aggregate stability, water conductivity, etc.) and to non-nutritional benefits such as improved biotic resistance of the plant (Newsham et al. 1995; Rillig 2005; Rillig et al. 2015). Comparatively, very little efforts have so far been invested into quantification of C fluxes in the mycorrhizal symbiosis, and to the underlying molecular mechanisms (Slavíková et al. 2017). The purpose of this chapter is to synthesize current knowledge on the influence of mycorrhiza on the C fluxes between atmosphere, plant, mycorrhizal fungi, and the soil. In this chapter, we focus mainly on the arbuscular mycorrhizal (AM) symbiosis, which is pertinent to most (>60%) plant species on Earth and also for most agricultural systems (Jemo et al. 2014; Sochorová et al. 2016), acknowledging similarities and differences between the different mycorrhizal types.

1.2 Magnitude of C Flow from Plants to the Mycorrhizal Fungi

Mycorrhizal fungi derive most of their C from their plant hosts, with only a little fraction (if any) of the C originating from the dead organic matter (Olsson and Johnson 2005; Hobbie et al. 2014; Lindahl and Tunlid 2015). Establishment of mycorrhizal symbiosis often increases allocation of C to the roots and further to the mycorrhizal fungi (Slavíková et al. 2017, and references therein), affecting whole plant C balance (Wright et al. 1998) and also the rate of plant photosynthesis, either directly through improved mineral nutrition or indirectly through increased below-ground C sink strength (Fig. 1.1, Douds et al. 2000; Kaschuk et al. 2009; Valentine et al. 2013). Due to the complexity of the interactions between the C and P economies (e.g., nutritional benefits conferred by the mycorrhizal association to the plant may stimulate host plant growth and thus C accumulation under nutrient limiting conditions to a great extent or completely compensate theoretical C allocation to the mycorrhizal fungus in a mycorrhizal plant of the same size as the nonmycorrhizal plant), there are different, partly contradicting concepts for calculation of mycorrhizal costs and benefits, sometimes resulting in conflicting

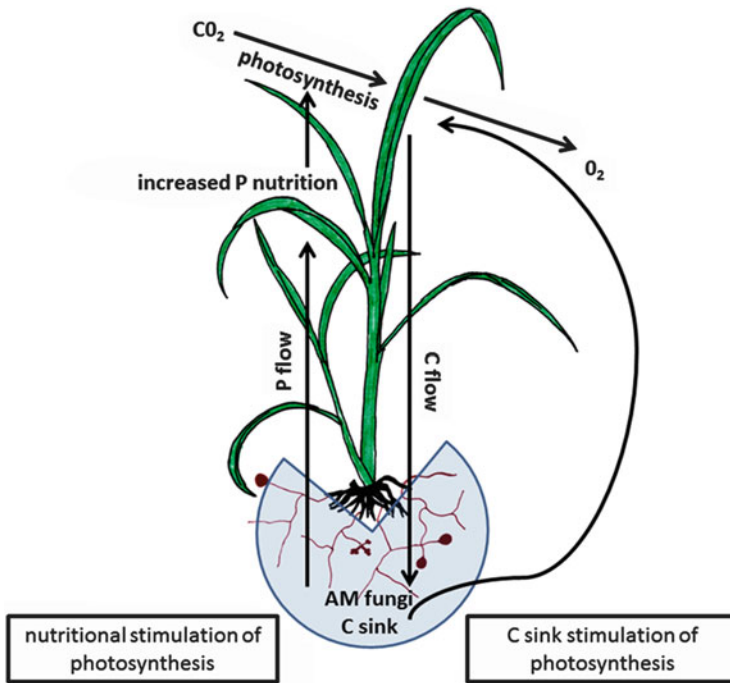


Fig. 1.1 Two possible pathways how establishment of arbuscular mycorrhiza could feed back on the rates/efficiency of photosynthesis of its plant host

predictions (Fitter 1991; Tinker et al. 1994; Landis and Fraser 2008; Correa et al. 2011).

In spite of the wealth of theories and predictions, the flux of C from the plant to the fungus could be quantified, particularly by employing isotopic C labeling, and relative C expenditure to mycorrhizas (e.g., the fraction of plant C budget allocated to the fungus) could be calculated from such data. Previously, mycorrhizal C cost of AM symbiosis was reported to reach between 4 and 20% of the photosynthetically fixed C by the plant (Smith and Read 2008). Yet, the value of 20% has only been recorded once for young cucumber plants under artificial environmental conditions (Jakobsen and Rosendahl 1990), but it has been frequently cited and also widely generalized up to a global ecosystem level (e.g., Brzostek et al. 2014). More recent research by Tomé et al. (2015) and by Slavíková et al. (2017) reported mycorrhizal C expenditure to reach only a few percent of the plant C budget (see Table 1.1 for more details), which is even below the previously reported low end (4%) of the C allocation to AM fungi. Yet, not all studies reported/measured C allocation to all relevant system compartments such as plant, soil, and the respiration losses above- and belowground. From the handful of studies including all relevant system compartments (coincidentally, all employing short-term pulse $^{14}\text{CO}_2$ labeling, Table 1.2), we learn that the shoot respiration could reach between 1 and 6%

Table 1.1 Mycorrhizal carbon (C) costs as a fraction of the total C budget of a host plant reported for various combinations of fungal and plant partners at different environmental contexts and assessed by different approaches

Reference	Plant-fungal partner combination			Length of labeling period	Length of chase period	Above-ground respiration assessed	Below-ground respiration assessed	Mycorrhizal cost (% of recorded C budget)	Note
	Host plant species	AM fungal species as reported	Current AM fungal name						
Pang and Paul (1980)	<i>Vicia faba</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	48 h	4.5 days	–	+	11 ^a	C in all measured compartments allocated to AM minus NM roots and belowground respiration
Paul and Kucey (1981)	<i>Vicia faba</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	48 h	96 h	+	+	4	Fraction of the whole assimilated C in mycorrhizal hyphae and fungal respiration
Kucey and Paul (1982)	<i>Vicia faba</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	48 or 8 h	96 or 116 h	+	+	3.5–4.2	C in all measured compartments allocated into mycorrhizal respiration and biomass
Snellgrove et al. (1982)	<i>Allium porrum</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	30 min	48 h	+	+	7	Total fixed C in roots of AM minus NM plants
Koch and Johnson (1984)	<i>Citrus aurantium</i> , <i>Poncirus trifoliata</i> × <i>Citrus sinensis</i>	<i>Glomus intraradices</i>	<i>Rhizophagus intraradices</i>	8.5 min	2 h	–	–	6–10	Difference of the total assimilated C to the half-roots between AM and NM parts in split-root system × 2

Harris et al. (1985)	<i>Glycine max</i>	<i>Glomus fasciculatum</i>	<i>Rhizophagus fasciculatus</i>	¹⁴ C	16 h	68 h	+	+	8–17	Total photosynthate allocated into AM biomass, AM respiration, root exudates + soil of AM plants (deduced from comparison of dually colonized (mycorrhizal + <i>Rhizobium</i>) vs. NM and NM + <i>Rhizobium</i> plants)
Douds et al. (1988)	<i>Poncirus trifoliata</i> × <i>Citrus sinensis</i>	<i>Glomus intraradices</i>	<i>Rhizophagus intraradices</i>	¹⁴ C	10 min	2 h	–	–	5.6–7.8	% assimilated C allocated to roots of AM minus NM plants
Wang et al. (1989)	<i>Panicum coloratum</i>	<i>Gigaspora margarita</i>		¹¹ C	100–120 min	200 min	–	–	>3.9	In the short-term study focused on ¹¹ C fluxes was not possible to calculate %C in all measured compartments. The authors quote that allocation to mycorrhizal part of the roots was probably more than 3.9% higher than to the nonmycorrhizal roots

(continued)

Table 1.1 (continued)

Reference	Plant-fungal partner combination			Length of labeling period	Length of chase period	Above-ground respiration assessed	Below-ground respiration assessed	Mycorrhizal cost (% of recorded C budget)	Note
	Host plant species	AM fungal species as reported	Current AM fungal name						
Jakobsen and Rosendahl (1990)	<i>Cucumis sativus</i>	<i>Glomus fasciculatum</i>	<i>Endogone arenacea</i>	16 h	80 h	+	+	20	% of assimilated C consumed by fungal biomass and its respiration
Peng et al. (1993)	<i>Citrus volkameriana</i>	<i>Glomus intraradices</i>	<i>Rhizophagus intraradices</i>			^b +	+	7 ^a	% C of the net C assimilation flow into root and soil respiration (AM minus NM)
Pearson and Jakobsen (1993)	<i>Cucumis sativus</i>	<i>Scutellospora calospora</i> , <i>Glomus caledonium</i> , <i>Glomus</i> sp.	<i>Scutellospora calospora</i> , <i>Funneliformis caledonium</i> , <i>Glomus</i> sp.	16 h	70 h	–	+	8.5–18.6 ^a	% of assimilated C allocated by AM minus NM plants to belowground (roots, ERM, belowground respiration)
Wright et al. (1998)	<i>Trifolium repens</i>	Field AM fungal community				^b +	+	15	% of the net amount of CO ₂ assimilated by AM plants respired by AM minus NM roots
Johnson et al. (2002a)	Grassland—24 plant species	Field AM fungal community		3.5 h	24 h	^d +	+	3.9–6.2	% of the fixed C passed through the ERM—no accumulation of ¹³ C observed in the substrate

Johnson et al. (2002b)	Grassland—24 plant species	Field AM fungal community		¹⁴ C	3 h	70 h	+ ^d	+	3.4	% C allocation of the photosynthetically fixed C by the plant into AM mycelium (incorporation into + release from AM fungi)
Grimoldi et al. (2006)	<i>Lolium perenne</i>	<i>Glomus hoi</i>		¹³ C	16 h	6–7 h	+	+	4.8–6	% C of daily gross photosynthesis allocated to the AM fungi
Heinemeyer et al. (2006)	<i>Plantago lanceolata</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	¹³ C	3.5 h	2 h	–	+	<1	% C of net photosynthesis allocated to ERM
Drigo et al. (2010)	<i>Festuca rubra</i>	Field AM fungal community		¹³ C	16 h	6 days	–	–	8.8–9 ^{ac}	% of total fixed C in the assessed compartments incorporated into the AM fungi (NLFA)
Lendenmann et al. (2011)	<i>Medicago truncatula</i>	<i>Glomus intraradices</i> , <i>Glomus claroideum</i> , <i>Gigaspora margarita</i>	<i>Rhizophagus intraradices</i> , <i>Claroideoglonus claroideum</i> , <i>Gigaspora margarita</i>	¹³ C	1 h	5 days	–	+	1.7–12.9 ^a	% C in all measured compartments allocated belowground (roots, substrate and belowground respiration), difference between AM and NM plants
Calderón et al. (2012)	<i>Sorghum bicolor</i>	<i>Glomus clarum</i>	<i>Rhizophagus clarus</i>	¹⁴ C	3 h	24 days	+	+	4 (6.8 ^a)	% photoassimilated C allocated belowground, difference between AM and NM plants

(continued)

Table 1.1 (continued)

Reference	Plant-fungal partner combination		Isotope	Length of labeling period	Length of chase period	Above-ground respiration assessed	Below-ground respiration assessed	Mycorrhizal cost (% of recorded C budget)	Note
	Host plant species	AM fungal species as reported							
Tomé et al. (2015)	<i>Fragaria ananassa</i> var. <i>elsanta</i>	Mix <i>Funneliformis mosseae</i> and <i>Rhizophagus intraradices</i>	¹³ C	6 h	1 and 7 days	–	–	1.8–4.3	% of total fixed C allocated to AM fungal mycelium
Slavíková et al. (2017)	<i>Medicago truncatula</i>	<i>Rhizophagus irregularis</i>	¹³ C	2 h	6 days	^b	+	2.3 (2.9)	% of the plant C budget allocated to the AM fungi—comparison between AM and NM plants of C allocation to substrate (or belowground)

Values were estimated with or without including above- and/or below-ground respiration

AM arbuscular mycorrhizal, NM non-mycorrhizal, ERM extraradical mycelium, NLFA neutral lipid fatty acid

^aOur calculation from the numbers provided in the publication

^bDark shoot respiration

^cApproximate values deduced from graphic presentation of results

^dApproximate figures of shoot respiration deduced from sequentially harvested pots

Table 1.2 Carbon (C) allocation into different compartments of the arbuscular mycorrhizal (AM) plant-soil system in studies assembling C budgets of the whole plants^a

Reference	Plant-fungal partner combination			Recently fixed C allocation (% of total)							AM fungus		Note	
	Host plant species	AM fungal species as reported	Current AM fungal name	Isotope	Length of labeling period	Length of chase period	Aboveground respiration	Shoot	Roots ^b	Substrate ^c	Belowground respiration ^d	AM fungal mycelia		AM fungal respiration
Paul and Kucey (1981)	<i>Vicia faba</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	¹⁴ C	48 h	96 h	2	40-47	18-19	0.5	28-31	1	3	A
Kucey and Paul (1982)	<i>Vicia faba</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	¹⁴ C	48 or 8 h	96 or 116 h	1-2.3	41.7-52	16.8-29		22.1-37.9	0.8-0.9	2.8-3.3	A
Snellgrove et al. (1982)	<i>Allium porrum</i>	<i>Glomus mosseae</i>	<i>Funneliformis mosseae</i>	¹⁴ C	30 min	48 h	2.3-6.3	49.7-60.8	15.7-27.1	2.1-5.3	9.7-23.1			A
Harris et al. (1985)	<i>Glycine max</i>	<i>Glomus fasciculatum</i>	<i>Rhizophagus fasciculatus</i>	¹⁴ C	16 h	68 h	4-6.3	51-61.2	9.9	1.3-1.8	14.6-16.3	2.7-2.8	4.7-13.7	B
Jakobsen and Rosendahl (1990)	<i>Cucumis sativus</i>	<i>Glomus fasciculatum</i>	<i>Rhizophagus fasciculatus</i>	¹⁴ C	16 h	80 h	2.5	54.1	13.2	2.3	27	0.8		A
Calderón et al. (2012)	<i>Sorghum bicolor</i>	<i>Glomus clarum</i>	<i>Rhizophagus clarus</i>	¹⁴ C	3 h	24 days	5	47.9	28.9	6.3	11.9			A

The studies vary in terms of symbiotic partner combinations, plant age, labeling pulse or chase periods, and presence or absence of *Rhizobia* for leguminous hosts

A—carbon allocation into the different compartments as reported by the authors, B—carbon allocation into the different compartments calculated by us from values provided by the authors

^aOnly including studies where all the relevant measurements were made and properly reported

^bIncluding nodules and intraradical mycelium for dually colonized leguminous hosts

^cIncluding extraradical AM fungal mycelium

^dIncluding rhizobial and fungal respiration if the latter is not explicitly provided

photosynthetically fixed C, C allocated to shoots 40–61%, C allocated to roots 10–29%, C allocated to substrate 1–6%, and C allocated specifically to AM fungal mycelium 1–3%; AM fungal respiration reaching 3–14%; and belowground respiration in total reaching 8–38% (Paul and Kucey 1981; Kucey and Paul 1982; Snellgrove et al. 1982; Harris et al. 1985; Jakobsen and Rosendahl 1990; Calderón et al. 2012).

Based on summary of all available literature on the magnitude of C fluxes in AM symbioses, it seems that the average C expenditure of the AM symbiosis may well be under 10% of the plant C budget (see Table 1.1 for more details). For comparison, in ectomycorrhizal symbioses, the magnitude of C allocated to fungal partner oscillates (apparently) around 3–36% of C fixed by photosynthesis (Bryla and Eissenstat 2005 and references therein). Very low (0.4% of the total C fixed by the plant) loss of plant photosynthate to its associated mycorrhizal fungus was, in contrast, reported for mycorrhizal green orchid *Goodyera repens* by Cameron et al. (2008).

The reported values on C allocation to AM fungi range widely. Here, the low number of publications dedicated to mycorrhizal C costs, especially in comparison with the quantity of literature concerning nutritional benefits of mycorrhizas, do not allow to properly uncover the determinants of plant C allocation to AM fungi. However, it seems that the choice of model host plant, AM fungal species and/or their combinations (Pearson and Jakobsen 1993; Lerat et al. 2003; Lendenmann et al. 2011), developmental stage of the symbiosis (Wright et al. 1998), environmental conditions (Slavíková et al. 2017), size and setup of the pots, and the duration of the isotope labeling/chase periods all strongly affect the outcome of quantification of C allocation to the AM fungi (see also Tables 1.1 and 1.2).

The exploration of mycorrhizal C cost has formerly been restricted by the available methodologies. Using ^{14}C radioisotope to directly trace the C fluxes from plant to mycorrhiza and to the soil was subject to strict health and radiosafety regulations (Schoor et al. 2016). Commercial availability of C sources enriched by stable ^{13}C isotope in the recent decades together with customization of the necessary mass spectrometry instrumentation made the direct C tracing much more available. However, despite the fact that the isotopic pulse-chase labeling enabled significant advances in assessing the C transfers within the plant–soil systems, it still only provides information with regard to the fate of recently fixed plant C, thus inevitably covering only a short period within the plant and/or fungal life cycles (Johnson 2008). This may be particularly short-sighted with respect to the mycorrhizal symbioses in trees and other long-living plants that could accumulate C reserves over long periods of time.

Further, the estimates of the mycorrhizal C costs based on incomplete C budgets (Pang and Paul 1980; Koch and Johnson 1984; Pearson and Jakobsen 1993; Heinemeyer et al. 2006; Drigo et al. 2010; Lendenmann et al. 2011) should be regarded with caution. This is because the gaseous C losses from shoots or roots/soil may reach a significant share of the plant C budget and thus should not be neglected (Lendenmann et al. 2011; Slavíková et al. 2017). Ignoring these C pools automatically leads to overestimation of the mycorrhizal C costs, which obviously was the case in some of the previous studies, although not the study by Jakobsen

and Rosendahl (1990) reporting the highest C costs of AM symbiosis ever (Table 1.1). Provided the rapidity of C fluxes between the plant, AM fungi, and the soil (Johnson 2008), it is sometimes very challenging to distinguish the C allocation to the root biomass, intra- and extraradical AM fungal mycelium and the soil/substrate, and to separate root and hyphal respirations (Heinemeyer et al. 2006). To this end, comparing mycorrhizal and nonmycorrhizal plants seems inevitable, although it is now widely accepted that this may be a source of many artifacts (Smith and Smith 2012). Moreover, depending on the balance between net costs and benefits of the symbiosis, mycorrhizal phenotypes appear to cover a whole continuum of plant responses to AM fungal colonization ranging from positive to neutral to negative (Johnson et al. 1997; Klironomos 2003). For some combinations of symbiotic partners and environmental conditions, mycorrhizal C costs may simply outweigh the growth benefits conferred to plants (Johnson et al. 2015), and it may not be possible to produce nonmycorrhizal and mycorrhizal plants of the same size and mineral nutrition (Peng et al. 1993; Graham et al. 1996; Lendenmann et al. 2011). Here, the solution to compare physiology of mycorrhizal and nonmycorrhizal plants may be in using P fertilization to produce mycorrhizal and nonmycorrhizal plants of the same size (Brown and Bethlenfalvai 1987; Baas and Lambers 1988; Slavíková et al. 2017). Another possibility is using plants with a split-root system (Douds et al. 2000).

Peripheral importance has been so far dedicated to fungus-to-plant C transfers, despite they have been shown as a significant component of the overall C budget (at least) in the orchid mycorrhizas. Yet, because up to 10% of plant species are at least partially mycoheterotrophic and receive a net C gain from their fungal symbiont for at least a part of their life (Leake 2005), they should be taken seriously. Clear demonstration of the fungus-to-plant C flux, although much lower than the C flow in opposite direction, was shown by Cameron et al. (2008) who quantified the bidirectional C fluxes by using ^{14}C labeling in green orchid *Goodyera repens* associated with fungus *Ceratobasidium cornigerum*. In ectomycorrhizas, the transfer of amino acid-C from fungus to plant has also been demonstrated (Abuzinadah and Read 1989), although importance of this mechanism for bulk C transfer from fungus to plant is probably low. Yet it may potentially have some impact on the C economy of the mycorrhizal symbiosis (Taylor et al. 2004) and thus should be incorporated in the assessments of mycorrhizal C cost. Such an “up-flow” of C may occur even in arbuscular and ericoid mycorrhizal associations, but have not been demonstrated as yet (Johnson 2008).

1.3 Mechanisms of C Transfer Between the Symbiotic Partners

Although it has been demonstrated many times that there is a fast and directed C transfer between the plants and the AM fungi (e.g., Johnson et al. 2002b; Dilkes et al. 2004; Olsson and Johnson 2005; Kiers et al. 2011), the molecular mechanisms

of such a transfer still remain elusive—no single gene responsible for mycorrhiza-directed C efflux from the plant cells specifically at the symbiotic interface has been identified as yet. This is in contrast to a wealth of knowledge on genes involved in the movement of sugars within a plant. The sugar fluxes from plant mesophyll (or sugar reserves elsewhere) to the rhizosphere are obviously coordinated by complex network involving many genes such as sucrose transporters, monosaccharide transporters (MSTs) and the SWEET translocator family (for more details see Doidy et al. 2012; Garcia et al. 2016). Indirect evidence makes the periarbuscular interface a hot candidate for site of exchange (trading) of C against the mineral nutrients, although unequivocal experimental proof for this is largely missing (Garcia et al. 2016). On the other hand, it seems well established that it is monosaccharides (particularly the glucose) that are the major form of C taken up by the AM fungi from the plant (Pfeffer et al. 1999; Schüßler et al. 2006; Nehls 2008; Helber et al. 2011). Thus, it seems that complex carbohydrates such as sucrose are cleaved at the plant–fungus interface, either by plant or fungal invertases (Casieri et al. 2013). Proton-ATPase activity on the plant membranes at the symbiotic interface indicates an active membrane transport mechanism (Krajinski et al. 2014), although direct coupling of this activity with C efflux has not yet been established. It seems, however, that the SWEET transporters are currently the hottest candidates for explaining the AM-directed C flux (Garcia et al. 2016).

On the fungal side, monosaccharide transporters were identified in ectomycorrhizal (Garcia et al. 2016 and references therein) as well as in AM fungi (Schüßler et al. 2006; Helber et al. 2011). First glomeromycotan MST (GpMST1) was described by Schüßler et al. (2006) in *Geosiphon pyriformis* interacting with autotrophic cyanobacterium *Nostoc punctiforme*. Its functional analogue in a “true” AM fungus appears to be the RiMST2 gene in *Rhizophagus irregularis*, which remains the only MST transporter proved so far to directly mediate sugar uptake by the AM fungus at the symbiotic interface with its plant host (Helber et al. 2011; Garcia et al. 2016).

1.4 Common Mycorrhizal Networks

Due to low host-mycobiont specificity in AM mycorrhizas, under most situations, the same AM fungal genotype at any given field site usually colonizes several plant individuals belonging to the same or different species. At the same time, the plant roots typically host multiple AM fungal species (Helgason et al. 2002; Vandenkoornhuysen et al. 2003), increasing the chances of sharing the mycobiont with the neighboring plant. The resulting “common mycorrhizal networks” (CMN) allow redistribution of mineral nutrients taken by the hyphae from the soil, water and the C costs between the involved plants (Merrild et al. 2013; Toju et al. 2013; Weremijewicz and Janos 2013; Prieto et al. 2016; Workman and Cruzan 2016) and also provide highways for transfer of signaling compounds between the plants (Johnson and Gilbert 2015). It is thus possible that one plant could feed the AM

hyphal network with C, whereas another plant would derive most of the benefits (mostly nutritional) of the shared association without providing much C to the fungus in return (Walder et al. 2012; Walder and van der Heijden 2015)—effectively resulting in “unfair” redistribution of the symbiotic C costs in the community. This could result in supporting the weaker competitor (e.g., a seedling vs. adult plant) or in strengthening the competition for resources, facilitated by the CMN (Newbery et al. 2000; Kytoviita et al. 2003; McGuire 2007; Weremijewicz and Janos 2013; Johnson 2015; Weremijewicz et al. 2016).

The big remaining question is, particularly with respect to the AM symbioses, whether any C could be transferred from the AM fungus to the plant. Although such a transfer has been postulated several years ago (Bidartondo et al. 2002; Simard and Durall 2004), current evidence for such a C transfer pathway is still equivocal, marked with a number of unanswered questions (Courty et al. 2011; Field et al. 2015) and opposing a strong experimental evidence that the fungus operates efficient control mechanisms (such as lipid/trehalose valve) ensuring the C to remain in the fungal tissues (Fitter et al. 1998; Pfeffer et al. 1999, 2004). This is in contrast to a well-established evidence about C transfer from the mycorrhizal fungus to its plant host in orchids in general and in achlorophyllous orchids in particular (Cameron et al. 2008; Barrett et al. 2010). What seems to be the rule, however, is that the mycorrhizal fungus obtains the C mainly or exclusively from a neighboring green plant rather than from the soil organic matter. This further reinforces the CMN as a key supply link to the mycoheterotrophic plants as well as in redistribution of C among ectomycorrhizal trees or shrubs (Selosse and Roy 2009; Deslippe and Simard 2011; Klein et al. 2016).

1.5 Food Chains

A substantial amount of C fixed by a plant is transported belowground shortly after photoassimilation and subsequently, within hours to days, detectable in the soil micro- and macrobes including the mycorrhizal fungi (Dilkes et al. 2004; Bahn et al. 2009; Mencuccini and Hölttä 2010). The mycorrhizal hyphae, who are responsible for diverting of up to several percent of plant photosynthetic production belowground (Table 1.1), then function as a specific channel for C flow from plant (leaves) down to soil (Kaiser et al. 2015), dictating who gets a share and who does not (Drigo et al. 2010; Schrey et al. 2015). The C from the mycorrhizal hyphae can pass onto the other members of soil biota as hyphal exudates. These could be produced either in an unspecific manner in form of organic acids or exoenzymes produced to the soil environment to manipulate nutrient availability in the immediate vicinity of the hyphae (Valentine et al. 2013; Sato et al. 2015) in a similar way as root exudates (Kuzyakov 2010; Bird et al. 2011; Philippot et al. 2013). Alternatively, hyphal exudates could be targeted to “hypersymbiotic” microbes associated with the hyphae and fulfilling specific and complementary functions to that of the hyphae themselves (Hodge et al. 2010; Cheng et al. 2012; Jansa et al. 2013; Taktek

et al. 2015; Zhang et al. 2016). Further, the C from the living or dead AM fungal hyphae/spores could also pass onto other soil biota by grazing/parasitism caused by a wide range of biotrophic microbes (Fitter and Garbaye 1994; Klironomos and Kendrick 1996; Rousseau et al. 1996; Crowther et al. 2012), and the mycorrhizal necromass is becoming a food source of soil saprotrophes (Treseder and Allen 2000). Worth mentioning remains the possible involvement of AM hyphal products such as glomalin in formation of recalcitrant soil organic matter—although the genesis and structure of this elusive compound is not entirely clear (Gadkar and Rillig 2006), it has been shown to correlate tightly with the presence/activity of AM fungi as well as with soil aggregate stability (Wright et al. 2006; Hammer and Rillig 2011; Fokom et al. 2013) and thus deserves a mention at this place—as potentially an important components of mycorrhizal C budget and also as a potential food source for specialized microbes.

1.6 Conclusions, Future Research Directions

The survey of literature on C fluxes in mycorrhizal plants (Table 1.1) offers few interesting insights: First, mycorrhizal C costs in terms of share of C fixed by the plant and allocated to the AM fungal symbiont is usually below 10%. This is an important observation since the maximum values (~20% of plant C budget) rather than the mean value (<10%) are most often cited in the secondary literature. Second, there is no observable increase in frequency of reports on C costs of mycorrhizal plants throughout the years. Since about 35 years, the papers appear sporadically, use different model systems and analytical approaches and our understanding of the determinants of the magnitude of C fluxes in mycorrhizal plants thus remain rather rudimentary, although we know lot more now than when Jack Harley published his “Problems of mycotrophy” in 1975 (Harley 1975). Third, not always have all the relevant compartments of the experimental system been included in the observations—most importantly, the aboveground respiration could represent 10 or more percent of the plant’s C budget and ignoring it could easily lead to overestimation of mycorrhizal C costs (Slavíková et al. 2017). Fourth, there is a large variation in the mycorrhizal C costs assignable to functional differences between symbiotic partners, environmental conditions, duration of the pulse-chase labeling, etc. (Table 1.1). It needs systematic efforts now to disentangle the determinants of magnitude/direction of C fluxes in mycorrhizal plants, which could eventually result in uncovering such important phenomena like the simultaneous bidirectional C fluxes in green orchid mycorrhizas (Cameron et al. 2008). The role of CMN and direction of C transfers in more complex plant–fungal communities should carefully be addressed in mycoheterotrophic plants, including achlorophyllous AM hosts, orchid, and Monotropoideae. To the best of our knowledge, there is also nearly no quantitative information on the magnitude of C fluxes between the partners in ericoid mycorrhiza, with a notable exception of a (qualitative) study by Grelet et al. (2009). Particularly, manipulation of light intensities in

model experiments is still rare, although they would directly be relevant to the C source strength of the mycorrhizal plants (Konvalinková and Jansa 2016).

It is also surprising how little is still known about the molecular mechanisms of C transfer between the symbiotic partners, despite the current wealth of high-throughput techniques and advanced tricks to study molecular design of living organisms. It seems like there is insufficient exchange of information and concepts between the molecular geneticists and the physiologists, although both are frequently approaching the same system and asking closely related questions. In this regard, it would be important to explore the possibilities of spatially explicit *in vivo* measurements of C transfers between symbiotic partners using ^{11}C -positron tomography (Wang et al. 1989) and possibly couple these with reporter genes or microRNA-based techniques to manipulate/visualize gene expression of specific genes.

Last but not least, the fact that ectomycorrhizal (and possibly also other mycorrhizal) fungi under field setting almost invariably rely on recently fixed C rather than on saprotrophy (Talbot et al. 2008; Lindahl and Tunlid 2015) is surprising given the ease of culturing some of these fungi on sugar-containing media *in vitro* (e.g., Hughes and Mitchell 1995; Midgley et al. 2004). It is hard to believe that the fungi would not use this capacity to obtain C saprotrophically at least under some circumstances. Indeed, more research in this direction is certainly warranted, particularly scrutinizing specific ecosystem scenarios conducive for expression of the saprotrophic capacity of the fungi—such as cold and wet periods, extensive period of darkness (polar nights), vegetation dormancy, or like.

Acknowledgment Research funding was provided by the Czech Science Foundation (project 14-19191S) and the Czech Ministry of Education, Youth and Sports (project No. LK11224). The authors also gratefully acknowledge further support from the Czech Academy of Sciences (J. E. Purkyně Fellowship to JJ) and the long-term research program RVO 61388971.

References

- Abuzinadah RA, Read DJ (1989) The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. 5. Nitrogen transfer in birch (*Betula pendula*) grown in association with mycorrhizal and non-mycorrhizal fungi. *New Phytol* 112:61–68
- Baas R, Lambers H (1988) Effects of vesicular-arbuscular mycorrhizal infection and phosphate on *Plantago major* ssp. *pleiosperma* in relation to the internal phosphate concentration. *Physiol Plant* 74:701–707
- Bahn M, Schmitt M, Siegwolf R, Richter A, Bruggemann N (2009) Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale? *New Phytol* 182:451–460
- Barrett CF, Freudenstein JV, Taylor DL, Koljalg U (2010) Rangeland analysis of fungal associations in the fully mycoheterotrophic *Corallorhiza striata* complex (Orchidaceae) reveals extreme specificity on ectomycorrhizal *Tomentella* (Thelephoraceae) across North America. *Am J Bot* 97:628–643

- Bever JD (2015) Preferential allocation, physio-evolutionary feedbacks, and the stability and environmental patterns of mutualism between plants and their root symbionts. *New Phytol* 205:1503–1514
- Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Dominguez L, Sersic A, Leake JR, Read DJ (2002) Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* 419:389–392
- Bird JA, Herman DJ, Firestone MK (2011) Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. *Soil Biol Biochem* 43:718–725
- Brown MS, Bethlenfalvay GJ (1987) *Glycine-Glomus-Rhizobium* symbiosis. 6. Photosynthesis in nodulated, mycorrhizal, or N-fertilized and P-fertilized soybean plants. *Plant Physiol* 85:120–123
- Bryla DR, Eissenstat DM (2005) Respiratory costs of mycorrhiza associations. In: Lambers H, Ribas-Carbo M (eds) *Plant respiration*. Springer, Dordrecht, pp 207–224
- Brzostek ER, Fisher JB, Phillips RP (2014) Modeling the carbon cost of plant nitrogen acquisition: Mycorrhizal trade-offs and multipath resistance uptake improve predictions of retranslocation. *J Geophys Res Biogeosci* 119:1684–1697
- Cairney JWG (2000) Evolution of mycorrhiza systems. *Naturwissenschaften* 87:467–475
- Calderón FJ, Schultz DJ, Paul EA (2012) Carbon allocation, belowground transfers, and lipid turnover in a plant-microbial association. *Soil Sci Soc Am J* 76:1614–1623
- Cameron DD, Johnson I, Read DJ, Leake JR (2008) Giving and receiving: measuring the carbon cost of mycorrhizas in the green orchid, *Goodyera repens*. *New Phytol* 180:176–184
- Casieri L, Lahmidi NA, Doïdy J, Veneault-Fourrey C, Migeon A, Bonneau L, Courty PE, Garcia K, Charbonnier M, Delteil A et al (2013) Biotrophic transportome in mutualistic plant-fungal interactions. *Mycorrhiza* 23:597–625
- Cavagnaro TR, Smith FA, Smith SE, Jakobsen I (2005) Functional diversity in arbuscular mycorrhizas: exploitation of soil patches with different phosphate enrichment differs among fungal species. *Plant Cell Environ* 28:642–650
- Cheng L, Booker FL, Tu C, Burkey KO, Zhou LS, Shew HD, Rufty TW, Hu SJ (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO₂. *Science* 337:1084–1087
- Correa A, Hampp R, Magel E, Martins-Loucao MA (2011) Carbon allocation in ectomycorrhizal plants at limited and optimal N supply: an attempt at unraveling conflicting theories. *Mycorrhiza* 21:35–51
- Courty PE, Walder F, Boller T, Ineichen K, Wiemken A, Rousteau A, Selosse MA (2011) Carbon and nitrogen metabolism in mycorrhizal networks and mycoheterotrophic plants of tropical forests: a stable isotope analysis. *Plant Physiol* 156:952–961
- Crowther TW, Boddy L, Jones TH (2012) Functional and ecological consequences of saprotrophic fungus-grazer interactions. *ISME J* 6:1992–2001
- Deslippe JR, Simard SW (2011) Below-ground carbon transfer among *Betula nana* may increase with warming in Arctic tundra. *New Phytol* 192:689–698
- Dilkes NB, Jones DL, Farrar J (2004) Temporal dynamics of carbon partitioning and rhizodeposition in wheat. *Plant Physiol* 134:706–715
- Doïdy J, Grace E, Kuhn C, Simon-Plas F, Casieri L, Wipf D (2012) Sugar transporters in plants and in their interactions with fungi. *Trends Plant Sci* 17:413–422
- Douds DD, Johnson CR, Koch KE (1988) Carbon cost of the fungal symbiont relative to net leaf-P accumulation in a split-root VA mycorrhizal symbiosis. *Plant Physiol* 86:491–496
- Douds DD, Pfeffer PE, Shachar-Hill Y (2000) Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In: Kapulnik Y, Douds DD (eds) *Arbuscular mycorrhizas: physiology and function*. Kluwer, Dordrecht, pp 107–129
- Drigo B, Pijl AS, Duyts H, Kielak A, Gamper HA, Houtekamer MJ, Boschker HTS, Bodelier PLE, Whiteley AS, van Veen JA et al (2010) Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO₂. *Proc Natl Acad Sci USA* 107:10938–10942

- Ferrol N, Barea JM, Azcón-Aguilar C (2002) Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas. *Plant Soil* 244:231–237
- Field KJ, Leake JR, Tille S, Allinson KE, Rimington WR, Bidartondo MI, Beerling DJ, Cameron DD (2015) From mycoheterotrophy to mutualism: mycorrhizal specificity and functioning in *Ophioglossum vulgatum* sporophytes. *New Phytol* 205:1492–1502
- Fitter AH (1991) Costs and benefits of mycorrhizas – implications for functioning under natural conditions. *Experientia* 47:350–355
- Fitter AH, Garbaye J (1994) Interactions between mycorrhizal fungi and other soil organisms. *Plant Soil* 159:123–132
- Fitter AH, Graves JD, Watkins NK, Robinson D, Scrimgeour C (1998) Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. *Funct Ecol* 12:406–412
- Fokom R, Mofor CT, Wakam LN, Megapche ELN, Tchameni S, Nwaga D, Rillig CM, Amvam PHA (2013) Glomalin, carbon, nitrogen and soil aggregate stability as affected by land use changes in the humid forest zone in South Cameroon. *Appl Ecol Environ Res* 11:581–592
- Gadkar V, Rillig MC (2006) The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. *FEMS Microbiol Lett* 263:93–101
- Garcia K, Doidy J, Zimmermann SD, Wipf D, Courty PE (2016) Take a trip through the plant and fungal transportome of mycorrhiza. *Trends Plant Sci* 21:937–950
- Graham JH, Drouillard DL, Hodge NC (1996) Carbon economy of sour orange in response to different *Glomus* spp. *Tree Physiol* 16:1023–1029
- Grelet GA, Johnson D, Paterson E, Anderson IC, Alexander IJ (2009) Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from *Piceirhiza bicolorata* ectomycorrhizas. *New Phytol* 182:359–366
- Grimoldi AA, Kavanová M, Lattanzi FA, Schaufele R, Schnyder H (2006) Arbuscular mycorrhizal colonization on carbon economy in perennial ryegrass: quantification by $^{13}\text{CO}_2/^{12}\text{CO}_2$ steady-state labelling and gas exchange. *New Phytol* 172:544–553
- Hammer EC, Rillig MC (2011) The influence of different stresses on glomalin levels in an arbuscular mycorrhizal fungus—salinity increases glomalin content. *PLoS ONE* 6(12): e28426. doi:10.1371/journal.pone.0028426
- Harley J (1975) Problems of mycotrophy. In: Sanders FE, Mosse B, Tinker PB (eds) *Endomycorrhizas*. Academic Press, London, pp 1–24
- Harris D, Pacovsky RS, Paul EA (1985) Carbon economy of soybean-*Rhizobium-Glomus* associations. *New Phytol* 101:427–440
- Heinemeyer A, Ineson P, Ostle N, Fitter AH (2006) Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytol* 171:159–170
- Helber N, Wipfel K, Sauer N, Schaarschmidt S, Hause B, Requena N (2011) A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp. is crucial for the symbiotic relationship with plants. *Plant Cell* 23:3812–3823
- Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *J Ecol* 90:371–384
- Hobbie EA, Hofmockel KS, Van Diepen LTA, Lilleskov EA, Ouimette AP, Finzi AC (2014) Fungal carbon sources in a pine forest: evidence from a ^{13}C -labeled global change experiment. *Fungal Ecol* 10:91–100
- Hodge A, Helgason T, Fitter AH (2010) Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecol* 3:267–273
- Hughes E, Mitchell DT (1995) Utilization of sucrose by *Hymenoscyphus ericae* (an ericoid endomycorrhizal fungus) and ectomycorrhizal fungi. *Mycol Res* 99:1233–1238
- Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytol* 115:77–83
- Jansa J, Bukovská P, Gryndler M (2013) Mycorrhizal hyphae as ecological niche for highly specialized hypsymbionts – or just soil free-riders? *Front Plant Sci* 4:134

- Jemo M, Souleymanou A, Frossard E, Jansa J (2014) Cropping enhances mycorrhizal benefits to maize in a tropical soil. *Soil Biol Biochem* 79:117–124
- Johnson D (2008) Resolving uncertainty in the carbon economy of mycorrhizal fungi. *New Phytol* 180:3–5
- Johnson D (2015) Priorities for research on priority effects. *New Phytol* 205:1375–1377
- Johnson D, Gilbert L (2015) Interplant signalling through hyphal networks. *New Phytol* 205:1448–1453
- Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol* 135:575–586
- Johnson D, Leake JR, Ostle N, Ineson P, Read DJ (2002a) In situ $^{13}\text{CO}_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytol* 153:327–334
- Johnson D, Leake JR, Read DJ (2002b) Transfer of recent photosynthate into mycorrhizal mycelium of an upland grassland: short-term respiratory losses and accumulation of ^{14}C . *Soil Biol Biochem* 34:1521–1524
- Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Booth RE, Grime JP, Young JPW, Read DJ (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytol* 161:503–515
- Johnson NC, Wilson GWT, Wilson JA, Miller RM, Bowker MA (2015) Mycorrhizal phenotypes and the Law of the Minimum. *New Phytol* 205:1473–1484
- Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, Solaiman ZM, Murphy DV (2015) Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. *New Phytol* 205:1537–1551
- Kaschuk G, Kuyper TW, Leffelaar PA, Hungria M, Giller KE (2009) Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biol Biochem* 41:1233–1244
- Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A et al (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333:880–882
- Klein T, Siegwolf RTW, Korner C (2016) Belowground carbon trade among tall trees in a temperate forest. *Science* 352:342–344
- Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84:2292–2301
- Klironomos JN, Kendrick WB (1996) Palatability of microfungi to soil arthropods in relation to the functioning of arbuscular mycorrhizae. *Biol Fertil Soils* 21:43–52
- Koch KE, Johnson CR (1984) Photosynthate partitioning in split-root *Citrus* seedlings with mycorrhizal and nonmycorrhizal root systems. *Plant Physiol* 75:26–30
- Konvalinková T, Jansa J (2016) Lights off for arbuscular mycorrhiza: on its symbiotic functioning under light deprivation. *Front Plant Sci* 7
- Krajinski F, Courty PE, Sieh D, Franken P, Zhang HQ, Bucher M, Gerlach N, Kryvoruchko I, Zoeller D, Udvardi M et al (2014) The H^+ -ATPase HA1 of *Medicago truncatula* is essential for phosphate transport and plant growth during arbuscular mycorrhizal symbiosis. *Plant Cell* 26:1808–1817
- Kucey RMN, Paul EA (1982) Carbon flow, photosynthesis, and N_2 fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.). *Soil Biol Biochem* 14:407–412
- Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. *Soil Biol Biochem* 42:1363–1371
- Kytoviita MM, Vestberg M, Tuom J (2003) A test of mutual aid in common mycorrhizal networks: established vegetation negates benefit in seedlings. *Ecology* 84:898–906
- Landis FC, Fraser LH (2008) A new model of carbon and phosphorus transfers in arbuscular mycorrhizas. *New Phytol* 177:466–479
- Leake JR (2005) Plants parasitic on fungi: unearthing the fungi in mycoheterotrophs and debunking the ‘Saprophytic’ plant myth. *Mycologist* 19:113–122

- Leake JR, Cameron DD (2010) Physiological ecology of mycoheterotrophy. *New Phytol* 185:601–605
- Lendenmann M, Thonar C, Barnard RL, Salmon Y, Werner RA, Frossard E, Jansa J (2011) Symbiont identity matters: carbon and phosphorus fluxes between *Medicago truncatula* and different arbuscular mycorrhizal fungi. *Mycorrhiza* 21:689–702
- Lerat S, Lapointe L, Gutjahr S, Piche Y, Vierheilig H (2003) Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent. *New Phytol* 157:589–595
- Lindahl BD, Tunlid A (2015) Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytol* 205:1443–1447
- McGuire KL (2007) Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. *Ecology* 88:567–574
- Mencuccini M, Hölttä T (2010) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phytol* 185:189–203
- Merrild MP, Ambus P, Rosendahl S, Jakobsen I (2013) Common arbuscular mycorrhizal networks amplify competition for phosphorus between seedlings and established plants. *New Phytol* 200:229–240
- Midgley DJ, Chambers SM, Cairney JWJ (2004) Utilisation of carbon substrates by multiple genotypes of ericoid mycorrhizal fungal endophytes from eastern Australian Ericaceae. *Mycorrhiza* 14:245–251
- Munkvold L, Kjølner R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytol* 164:357–364
- Nehls U (2008) Mastering ectomycorrhizal symbiosis: the impact of carbohydrates. *J Exp Bot* 59:1097–1108
- Newbery DM, Alexander IJ, Rother JA (2000) Does proximity to conspecific adults influence the establishment of ectomycorrhizal trees in rain forest? *New Phytol* 147:401–409
- Newsham KK, Fitter AH, Watkinson AR (1995) Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J Ecol* 83:991–1000
- Nguyen NH, Song ZW, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol* 20:241–248
- Olsson PA, Johnson NC (2005) Tracking carbon from the atmosphere to the rhizosphere. *Ecol Lett* 8:1264–1270
- Pang PC, Paul EA (1980) Effects of vesicular-arbuscular mycorrhiza on ^{14}C and ^{15}N distribution in nodulated fababeans. *Can J Soil Sci* 60:241–250
- Paul EA, Kucey RMN (1981) Carbon flow in plant microbial associations. *Science* 213:473–474
- Pearson JN, Jakobsen I (1993) Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. *New Phytol* 124:481–488
- Peng SB, Eissenstat DM, Graham JH, Williams K, Hodge NC (1993) Growth depression in mycorrhizal *Citrus* at high phosphorus supply – analysis of carbon costs. *Plant Physiol* 101:1063–1071
- Pfeffer PE, Douds DD, Bécard G, Shachar-Hill Y (1999) Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. *Plant Physiol* 120:587–598
- Pfeffer PE, Douds DD, Bücking H, Schwartz DP, Shachar-Hill Y (2004) The fungus does not transfer carbon to or between roots in an arbuscular mycorrhizal symbiosis. *New Phytol* 163:617–627
- Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11:789–799
- Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) *Mycorrhiza*, Springer, Switzerland, pp 1–7

- Prieto I, Roldán A, Huygens D, Alguacil MD, Navarro-Cano JA, Querejeta JI (2016) Species-specific roles of ectomycorrhizal fungi in facilitating interplant transfer of hydraulically redistributed water between *Pinus halepensis* saplings and seedlings. *Plant Soil* 406:15–27
- Rillig MC (2005) A connection between fungal hydrophobins and soil water repellency? *Pedobiologia* 49:395–399
- Rillig MC, Aguilar-Trigueros CA, Bergmann J, Verbruggen E, Veresoglou SD, Lehmann A (2015) Plant root and mycorrhizal fungal traits for understanding soil aggregation. *New Phytol* 205:1385–1388
- Rousseau A, Benhamou N, Chet I, Piche Y (1996) Mycoparasitism of the extramatrical phase of *Glomus intraradices* by *Trichoderma harzianum*. *Phytopathology* 86:434–443
- Sato T, Ezawa T, Cheng WG, Tawarayama K (2015) Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus *Rhizophagus clarus*. *Soil Sci Plant Nutr* 61:269–274
- Schrey SD, Hartmann A, Hampp R. (2015) Rhizosphere interactions. In: *Ecological biochemistry: environmental and interspecies interactions*. Wiley, Weinheim, pp 293–310
- Schuur EAG, Carbone MS, Hicks Pries CE, Hopkins FM, Natali SM (2016) Radiocarbon in terrestrial ecosystems. In: EAG S, ERM D, Trumbore SE (eds) *Radiocarbon and climate change. Mechanisms, applications and laboratory techniques*. Springer, Cham, pp 167–220
- Schüßler A, Martin H, Cohen D, Fitz M, Wipf D (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. *Nature* 444:933–936
- Selosse MA, Roy M (2009) Green plants that feed on fungi: facts and questions about mixotrophy. *Trends Plant Sci* 14:64–70
- Simard SW, Durall DM (2004) Mycorrhizal networks: a review of their extent, function, and importance. *Can J Bot* 82:1140–1165
- Slavíková R, Püschel D, Janoušková M, Hujšlová M, Konvalinková T, Gryndlerová H, Gryndler M, Weiser M, Jansa J (2017) Monitoring CO₂ emissions to gain a dynamic view of carbon allocation to arbuscular mycorrhizal fungi. *Mycorrhiza* 27:35–51
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic Press, London
- Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* 104:1–13
- Snellgrove RC, Splittstoesser WE, Stribley DP, Tinker PB (1982) The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular arbuscular mycorrhizas. *New Phytol* 92:75–87
- Sochorová L, Jansa J, Verbruggen E, Hejčman M, Schellberg J, Kiers ET, Johnson NC (2016) Long-term agricultural management maximizing hay production can significantly reduce belowground C storage. *Agric Ecosyst Environ* 220:104–114
- Taktek S, Trepanier M, Servin PM, St-Arnaud M, Piche Y, Fortin JA, Antoun H (2015) Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* DAOM 197198. *Soil Biol Biochem* 90:1–9
- Talbot JM, Allison SD, Treseder KK (2008) Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Funct Ecol* 22:955–963
- Taylor AFS, Gebauer G, Read DJ (2004) Uptake of nitrogen and carbon from double-labelled (¹⁵N and ¹³C) glycine by mycorrhizal pine seedlings. *New Phytol* 164:383–388
- Tinker PB, Durall DM, Jones MD (1994) Carbon use efficiency in mycorrhizas – theory and sample calculations. *New Phytol* 128:115–122
- Toju H, Sato H, Yamamoto S, Kadowaki K, Tanabe AS, Yazawa S, Nishimura O, Agata K (2013) How are plant and fungal communities linked to each other in belowground ecosystems? A massively parallel pyrosequencing analysis of the association specificity of root-associated fungi and their host plants. *Ecol Evol* 3:3112–3124
- Tomé E, Tagliavini M, Scandellari F (2015) Recently fixed carbon allocation in strawberry plants and concurrent inorganic nitrogen uptake through arbuscular mycorrhizal fungi. *J Plant Physiol* 179:83–89
- Treseder KK, Allen MF (2000) Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO₂ and nitrogen deposition. *New Phytol* 147:189–200

- Valentine AJ, Mortimer PE, Kleinert A, Kang Y, Benedito VA (2013) Carbon metabolism and costs of arbuscular mycorrhizal associations to host roots. *Symbiotic Endophytes* 37:233–252
- van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72
- Vandenkoornhuysen P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Mol Ecol* 12:3085–3095
- Walder F, van der Heijden MGA (2015) Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. *Nat Plants* 1(11). doi:[10.1038/nplants.2015.159](https://doi.org/10.1038/nplants.2015.159)
- Walder F, Niemann H, Natarajan M, Lehmann MF, Boller T, Wiemken A (2012) Mycorrhizal networks: common goods of plants shared under unequal terms of trade. *Plant Physiol* 159:789–797
- Wang GM, Coleman DC, Freckman DW, Dyer MI, McNaughton SJ, Acra MA, Goeschl JD (1989) Carbon partitioning patterns of mycorrhizal versus non-mycorrhizal plants – real-time dynamic measurements using ^{14}C . *New Phytol* 112:489–493
- Weremijewicz J, Janos DP (2013) Common mycorrhizal networks amplify size inequality in *Andropogon gerardii* monocultures. *New Phytol* 198:203–213
- Weremijewicz J, da Silveira Lobo O'Reilly Sternberg L, Janos DP (2016) Common mycorrhizal networks amplify competition by preferential mineral nutrient allocation to large host plants. *New Phytol* 212:461–471
- Workman RE, Cruzan MB (2016) Common mycelial networks impact competition in an invasive grass. *Am J Bot* 103:1041–1049
- Wright DP, Read DJ, Scholes JD (1998) Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant Cell Environ* 21:881–891
- Wright SF, Nichols KA, Schmidt WF (2006) Comparison of efficacy of three extractants to solubilize glomalin on hyphae and in soil. *Chemosphere* 64:1219–1224
- Zhang L, Xu MG, Liu Y, Zhang FS, Hodge A, Feng G (2016) Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytol* 210:1022–1032

Chapter 2

Basic and Applied Research for Desert Truffle Cultivation

Asunción Morte, Manuela Pérez-Gilabert, Almudena Gutiérrez,
Francisco Arenas, José Eduardo Marqués-Gálvez, Juan Julián Bordallo,
Antonio Rodríguez, Luis Miguel Berná, Cecilia Lozano-Carrillo,
and Alfonso Navarro-Ródenas

Abstract This chapter summarizes the latest basic and applied advances in desert truffle research carried out to improve our knowledge of the biodiversity, physiology, biotechnology, and cultivation of these hypogeous and edible fungi. ITS-rDNA sequences in phylo-geographic studies and host plant and soil pH characteristics have been the key to describing eight new desert truffle species. The production of desert truffle mycorrhizal plants has been improved by using β -cyclodextrin and bioreactors for mycelium culture and native beneficial bacteria (PGPR and MHB) to increase seedling survival and mycorrhization. Some fungal enzymes have also been characterized in *Terfezia claveryi* ascocarps. The presence of alkaline phosphatase both in mycelia and ascocarps indicates that this enzyme plays an important role during the life cycle of *T. claveryi*, while acid phosphatase might be involved in a process that takes place during the ascocarp stage. Numerous desert truffle plantations have been established in Spain in the last 10 years. A high density of mycorrhizal plants combined with a proper irrigation are two important factors to stimulate ascocarp production. The combination of a high rate of intracellular colonization together with the fine-tuned expression of fungal and plant aquaporins could result in a morpho-physiological adaptation of this symbiosis in drought conditions. Moreover, desert truffle silviculture is proposed for improving truffle production and for conserving the natural areas where desert truffle grow.

A. Morte (✉) • A. Gutiérrez • F. Arenas • J.E. Marqués-Gálvez • J.J. Bordallo • A. Rodríguez • L.M. Berná • C. Lozano-Carrillo • A. Navarro-Ródenas
Dpto. Biología Vegetal (Botánica), Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain
e-mail: amorte@um.es

M. Pérez-Gilabert
Dpto. Bioquímica y Biología Molecular-A, Facultad de Biología, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain

2.1 Introduction

The first plantation of the desert truffle *Terfezia claveryi* was established in 1999 in south-east Spain (Murcia) (Honrubia et al. 2001), since when most of the data related to the biotechnological aspects of the production of mycorrhizal plants and plantation management practices have been compiled in three publications of Springer (Morte et al. 2008, 2009, 2012). More recently, additional information on desert truffles related to soil properties (Bonifacio and Morte 2014), the types of mycorrhiza (Roth-Bejerano et al. 2014), cryptic and new species (Bordallo and Rodriguez 2014), the benefits conferred on plants (Kagan-Zur et al. 2014a), ascocarp enzymes (Pérez-Gilabert et al. 2014), and cultivation (Morte and Andriano 2014; Honrubia et al. 2014) have been published by our group in the first international and monographic book devoted to desert truffles by the same publisher (Kagan-Zur et al. 2014b). However, the increasing demand for this crop, in Spain and in other countries, has prompted a search for new strategies to increase ascocarp production in the field, to improve the production protocol of mycorrhizal plants, and to advance our knowledge of the biology and biodiversity of these desert truffles.

The present chapter describes all the experiments carried out to obtain these objectives and the last results obtained.

2.2 Edible and New Species of Desert Truffles

The edible hypogeous ascomata of fungi belonging mostly to the Pezizaceae family are known as “desert truffles” due to their habitat, typically arid and semi-arid ecosystems, mostly in the Mediterranean region (Morte et al. 2009; Zambonelli et al. 2014), where they constitute an important economic resource for local populations. Species of *Terfezia* and *Tirmania* have a long history of culinary and medical use because they are rich in nutrients and bioactive compounds (Shavit 2014).

Recently, some new species of desert truffles have been identified (Bordallo et al. 2012, 2013, 2015; Kovács et al. 2011). The ITS-rDNA sequence, host plant, and soil pH seem to be the key to describing new desert truffle species. *Terfezia* species (or their host) seem to be able to adapt to a wide range of soil pH values (high or relatively low), edaphic conditions, and texture (Bonifacio and Morte 2014). *Terfezia canariensis* has been described as belonging to the claveryi group. Five new *Terfezia* species—*T. pini*, *T. pseudoleptoderma*, *T. albida*, *T. grisea*, and *T. cistophyla*—have recently been proposed as forming part of the previously single leptoderma–olbiensis cryptic group, and another two species, *T. eliocrocae* from alkaline soils and *T. extremadurensis* from acid soils, have also been proposed as new species (Fig. 2.1). In desert truffles, as in the case of mycorrhizal fungi, the preference-specificity factor of the host is regarded as an

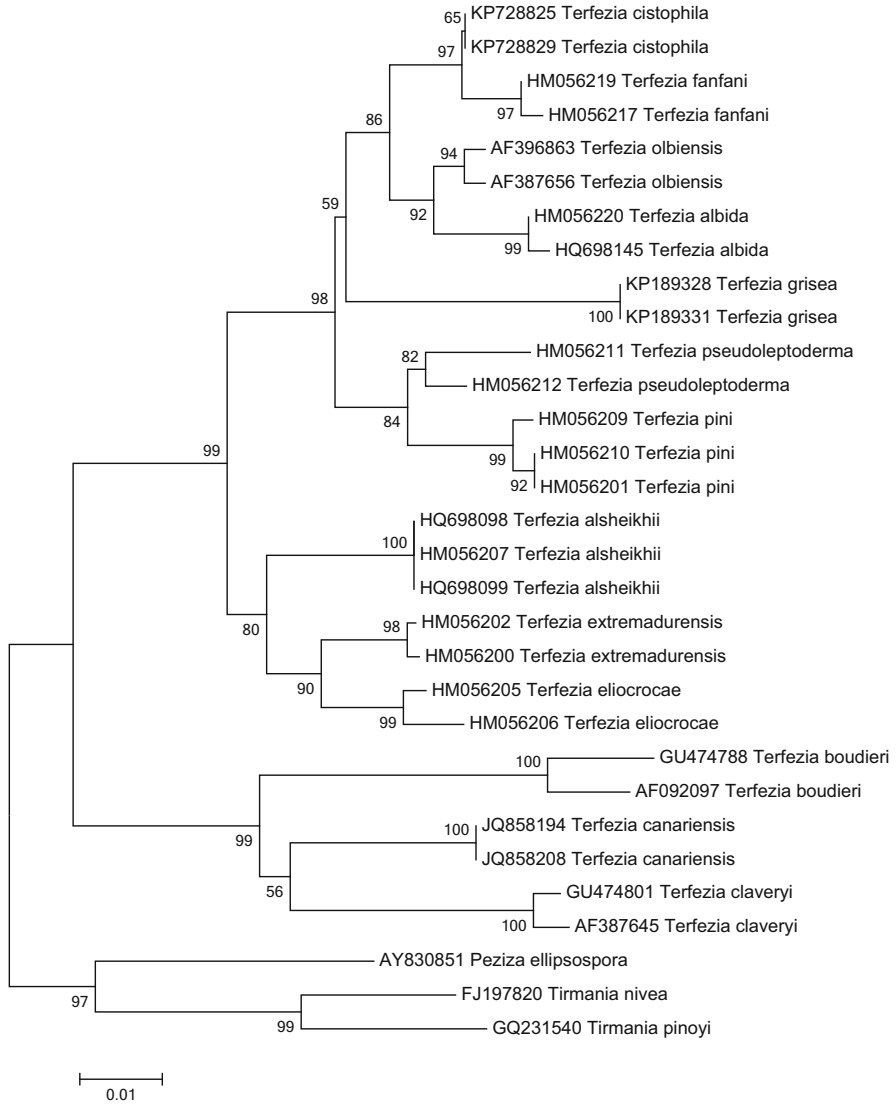


Fig. 2.1 The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset. There were a total of 400 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4

important factor for understanding their life cycle. The difficulty of sampling desert truffles implies their slow discovery (Bordallo and Rodríguez 2014). However, using new tools, such as molecular biology and phylo-geographic studies, should allow us to identify differences among cryptic species.

However, not all hypogeous species belonging to the desert truffle genera are edible fungi. So, what makes a desert truffle edible? Apart from their taste, the nutrients, and antioxidant compounds they contain, a medium-large size and an easy to clean peridium are essential if they are harvested and marketed. For example, due to its small size and pubescent peridium that accumulates soil debris, *Picoa lefebvrei* (Pat.) Maire is not marketed despite it being especially rich in natural antioxidants (Murcia et al. 2002) and very tasty, much more so than many edible *Terfezia* species.

At least, 14 edible desert truffles have been identified as being regularly consumed by people (Table 2.1), all of which have characteristic host plants and soil pHs that define their mycorrhizal symbiosis and ecology (Table 2.1).

Table 2.1 Edible species of desert truffles, their host plant species, and soil pH requirements

Edible desert truffles	Host plants	Soil pH
<i>Choiromyces magnusii</i>	<i>Cistus ladanifer</i>	Acid
<i>Mattiolomyces terfezioides</i>	<i>Citrus</i> spp, <i>Prunus</i> spp <i>Helianthemum ovatum</i> <i>Robinia pseudoacacia</i>	Alkaline–neutral
<i>Picoa juniperi</i>	<i>Helianthemum</i> spp	Alkaline
<i>Picoa lefebvrei</i>	<i>Helianthemum</i> spp	Alkaline
<i>Terfezia arenaria</i>	<i>Tuberaria guttata</i>	Acid
<i>Terfezia boudieri</i>	<i>Helianthemum lippii</i> <i>Helianthemum salicifolium</i> <i>Helianthemum sessiliflorum</i>	Alkaline
<i>Terfezia canariensis</i>	<i>Helianthemum canariense</i>	Alkaline
<i>Terfezia claveryi</i>	<i>Helianthemum almeriense</i> <i>Helianthemum canariense</i> <i>Helianthemum guttatum</i> <i>Helianthemum hirtum</i> <i>Helianthemum ledifolium</i> <i>Helianthemum salicifolium</i> <i>Helianthemum violaceum</i>	Alkaline
<i>Terfezia fanfani</i>	<i>Tuberaria guttata</i>	Acid
<i>Terfezia leptoderma</i>	<i>Helianthemum salicifolium</i> <i>Tuberaria guttata</i>	Acid
<i>Tirmania nivea</i>	<i>Helianthemum salicifolium</i> <i>Helianthemum lippii</i>	Alkaline
<i>Tirmania pinoyi</i>	<i>Helianthemum salicifolium</i> <i>Helianthemum lippii</i>	Alkaline
<i>Tuber lacunosum</i>	<i>Tuberaria guttata</i>	Acid
<i>Tuber oligospermum</i>	<i>Pinus</i> spp, <i>Quercus</i> spp, <i>Cistus</i> spp	Acid–alkaline

Among these fungal species, two species of desert truffle have been successfully cultivated and reported, *Terfezia claveryi* Chatin in Spain (Honrubia et al. 2001; Morte et al. 2008, 2009, 2010, 2012) and *Terfezia boudieri* Chatin in Tunisia (Slama et al. 2010) and Israel (Khagan-Zur, pers. com.). More recently, mycorrhizal plants with *Picoa lefebvrei* and *Tirmania nivea* have been planted in 2014, and with *Terfezia arenaria* in 2015, in Spain, but fruiting ascocarps have not yet been obtained.

2.3 Production of Desert Truffle Mycorrhizal Plants

The increasing demand for desert truffle mycorrhizal plants for desert truffle cultivation has prompted research into new strategies to help the transition from experimental scale to medium-large scale cultivation. The first step in this process is the selection and production of suitable mycorrhizal seedlings of good quality and adapted to different cultivation sites.

For the mycorrhizal synthesis, both seedlings and micropropagated plants of *Helianthemum* species, together with *Terfezia* spores and mycelium, have been used (Morte and Andrino 2014; Morte et al. 2008, 2009). The system for mycorrhizal plant production from *Helianthemum* seeds and *Terfezia* spores is the most used because it is cheaper than using micropropagated plants and mycelium as inoculum. However, each of these systems presents its own strengths and weaknesses.

2.3.1 Plant Production

For this purpose, a suitable host plant species should be chosen, taking into account edaphic and bioclimatic conditions; if possible, it is better to use a perennial rather than an annual species.

Most of the plant species reported as host plants for experimental desert truffle mycorrhization are perennial and annual species from *Helianthemum* genus, belonging to the Cistaceae (Morte and Andrino 2014). Many *Helianthemum* species show erratic seed germination, and seed scarification is necessary to increase germination rates. Moreover, high mortality of the germinated seedlings is common during the first 2 months after germination in nursery conditions (Morte et al. 2012). Micropropagation techniques have been used for plant production since they improve seed germination and plant survival (Morte et al. 2008). *Helianthemum almeriense* Pau has been successfully micropropagated by the photomixoautotrophic (PM) method (Morte and Honrubia 1992, 1997), and the same plant was used as a model to improve *Helianthemum* propagation by photoautotrophic (PA) micropropagation (Morte et al. 2012). When cultured in the absence of sucrose, this plant increased its survival rate during acclimation using

a PA system. At the same time, substituting agar by perlite, as physical support, contributed to a significant reduction in plant losses during acclimation. In addition, the absence of sucrose reduced the presence of microbial contamination during the cultivation vessel phase (Morte et al. 2012). This method permitted us to grow a large volume of *H. almeriense* seedlings with germination rates of around 80–90% and very satisfactory results.

To ascertain the most suitable moment for plant transplantation from in vitro to ex vitro conditions in order to prevent plant losses, the probability of plant survival was estimated based only on a chlorophyll meter SPAD-502 measurements. The maximum survival rate for *H. almeriense* was established at 28 SPAD-502 units, or its equivalent in total chlorophyll content, 1.6 mg/g leaf (Morte and Andrino 2014).

In addition to plant micropropagation, we tried to improve plant production from *Helianthemum* seeds by reducing the high mortality of the germinated seedlings that normally occurs during the first 4 weeks after germination in nursery conditions (Morte et al. 2008). It was realized that the early inoculation of *Helianthemum* seedlings with *Terfezia* was not sufficient to enhance the low survival rates in nursery conditions. This led us to wonder if the use of other microorganisms present in the rhizosphere of the mycorrhizal association *Helianthemum* × *Terfezia*, such as plant growth-promoting rhizobacteria (PGPR), could help in one or more stages of mycorrhizal plant production system. For this purpose, 64 native bacterial colonies were isolated from mycorrhizal roots of *H. almeriense* with *T. claveryi*, mycorrhizosphere soil, and peridium of *T. claveryi* in order to evaluate their effect on the mycorrhizal plant production (Navarro-Ródenas et al. 2016). Based on a phylogenetic analysis of the 16S rDNA partial sequence, the 64 colonies were gathered in 45 different strains from 17 genera, the largest genera being *Pseudomonas* (41% of the isolated strains), *Bacillus* (12% of the isolated strains), and *Varivorax* (8%). All bacteria were characterized phenotypically and by their PGPR traits, including auxin and siderophore production, phosphate solubilization, and the presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Table 2.2). Only bacterial combinations with several PGPR traits and *Pseudomonas fluorescens*, strain 5, which presents three different PGPR traits, showed a positive effect on plant survival and growth. Particularly relevant were the bacterial treatments involving auxin release, which significantly increased the root–shoot ratio and the mycorrhizal percentage (Table 2.3). Moreover, *Pseudomonas mandelii* strain 29 was able to considerably increase the mycorrhization ratio but not plant growth, thus being considered as a mycorrhiza helper bacterium (Table 2.3). Among these bacteria, the fluorescent pseudomonads complex was the most abundant and significant in terms of the effects on PGPR traits in the *Terfezia* × *Helianthemum* symbiosis. The use of some of these bacteria at different stages of nursery plant production helps overcome some of the current bottlenecks of desert truffle plant production at semi-industrial scale. The benefits would include increased survival rates and mycorrhization, reduced production time, and, ultimately, greater plant quality (Navarro-Ródenas et al. 2016). Therefore, the mycorrhizal roots, mycorrhizosphere soil, and peridium of desert truffles must be regarded

Table 2.2 Characterization of plant growth-promoting traits

Strain n°	Organisms identified	Phosphate solubilization ^a	IAA production ^b	Siderophore production ^c	ACC desaminase
1	<i>Pseudomonas</i> sp.	++	–	+	–
2	<i>Paenibacillus</i> sp.	–	–	–	–
3	<i>Bacillus thuringiensis</i>	–	–	–	–
4	<i>Achromobacter</i> sp.	–	–	–	–
5	<i>Pseudomonas fluorescens</i>	++	+	–	+
6	<i>Microbacterium paraoxydans</i>	–	+	–	–
7	<i>Pseudomonas</i> sp.	–	++	++	–
8	<i>Bacillus atrophaeus</i>	–	–	–	–
9	<i>Pseudomonas</i> sp.	–	–	–	–
10	<i>Pseudomonas</i> sp.	++	–	+	–
11	<i>Bacillus megaterium</i>	++	–	–	–
12	<i>Sphingomonas</i> sp.	–	–	–	–
13	<i>Rhizobium radiobacter</i>	–	–	–	–
14	<i>Acinetobacter lwoffii</i>	–	–	–	–
15	<i>Flavobacterium</i> sp.	–	–	++	–
16	<i>Novosphingobium panipatense</i>	–	+	–	–
17	<i>Bacillus simplex</i>	–	–	++	–
18	<i>Stenotrophomonas rhizophila</i>	–	–	–	–
19	<i>Arthrobacter</i> sp.	–	–	–	–
20	<i>Sinorhizobium meliloti</i>	–	–	–	–
21	<i>Pseudomonas</i> sp.	++	–	++	–
22	<i>Variovorax paradoxus</i>	–	–	–	–
23	<i>Variovorax paradoxus</i>	–	–	–	–
24	<i>Phyllobacterium bourgognense</i>	–	–	–	–
25	<i>Pseudomonas</i> sp.	–	–	–	–
26	<i>Microvirga</i> sp.	–	+	–	–
27	<i>Pseudomonas</i> sp.	–	–	–	–
28	<i>Pseudomonas moraviensis</i>	–	–	+	–
29	<i>Pseudomonas mandelii</i>	++	–	–	–
30	<i>Pseudomonas</i> sp.	–	–	–	–

(continued)

Table 2.2 (continued)

Strain n°	Organisms identified	Phosphate solubilization ^a	IAA production ^b	Siderophore production ^c	ACC desaminase
31	<i>Pseudomonas</i> sp.	–	–	–	–
32	<i>Pseudomonas</i> sp.	–	–	–	–
33	<i>Pseudomonas</i> sp.	–	–	+	–
34	<i>Pseudomonas brenneri</i>	+++	–	+	–
35	<i>Rhodococcus</i> sp.	–	–	–	–
36	<i>Flavobacterium</i> sp.	–	–	–	–
37	<i>Phyllobacterium ifriqiyense</i>	–	–	–	–
38	<i>Variovorax paradoxus</i>	–	–	–	–
39	<i>Rhizobium galegae</i>	–	–	–	–
40	<i>Pseudomonas</i> sp.	++	+	–	–
41	<i>Arthrobacter nitroguajacolicus</i>	–	–	–	–
42	<i>Pseudomonas</i> sp.	+	–	+++	–
43	<i>Arthrobacter</i> sp.	–	+++	–	–
44	<i>Pseudomonas</i> sp.	–	–	–	–
45	<i>Variovorax paradoxus</i>	–	–	–	–

Modified from Navarro-Ródenas et al. (2016)

^a+ values <300 µg/mL, ++ values >300–550 µg/mL, +++ values >550 µg/mL

^b+ values <50 µg/mL, ++ values >50–100 µg/mL, +++ values >100 µg/mL

^c+ values <20 µg/mL, ++ values >20–60 µg/mL, +++ values >60 µg/mL

as environments enriched in bacteria which can increase the quality of the plant in the desert truffle plant production system at semi-industrial scale.

2.3.2 Fungal Inoculum Production

Mycorrhizal plants have been successfully produced by using both desert truffle spores and mycelia (Morte et al. 2008). Mature spores, obtained by blending truffles, are the most commonly used due to the slow and erratic growth of the mycelium in vitro. Working with spore inoculum, the spore solution can be applied directly to the plants or using perlite as a carrier, whereby the spores adhere to the pores and cavities within (Andrino et al. 2012; Morte et al. 2012). Using such a carrier technique allowed us to use 40% fewer of spores (Morte and Andrino 2014). However, the problem with spore inoculation techniques is that pests, pathogens, and other mycorrhizal fungi can still contaminate the plants (Iotti et al. 2016).

Table 2.3 Summary of the effects of the different bacterial treatments on plant bioassays at different stages of mycorrhizal plant development

	Treatment	Stages			
		i	ii	iii	
Control	Species	Survival	Growth	Root/shoot ratio	Mycorrhization
5	<i>Ps. fluorescens</i>	*	N	**	**
15	<i>Flavobacterium</i> sp	N	N	*	N
29	<i>Ps. mandelii</i>	N	N	N	***
44	<i>Arthrobacter</i> sp	N	N	***	**
5+7	<i>Ps. fluorescens</i> + <i>Pseudomonas</i> sp	***	N	*	*
34+7	<i>Ps. brenneri</i> + <i>Pseudomonas</i> sp	N	N	*	*
15+41	<i>Flavobacterium</i> sp+ <i>Pseudomonas</i> sp	***	N	N	***
44+21	<i>Arthrobacter</i> sp+ <i>Pseudomonas</i> sp	**	N	N	N

N absence of significance (modified from Navarro-Ródenas et al. 2016)

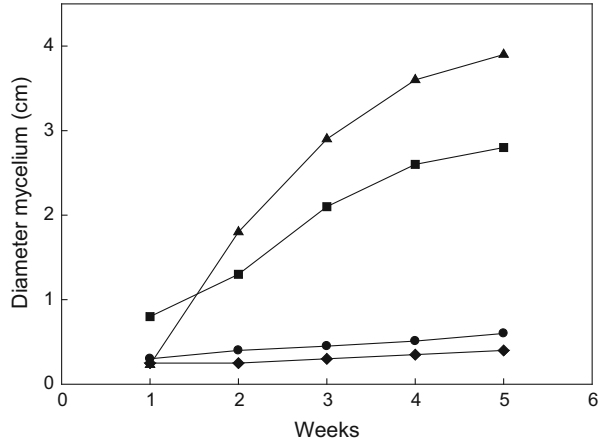
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, level of significance with regard to the control

Therefore, it is more advisable to use mycelium than spores whenever possible and profitable.

Desert truffle mycelia have been grown successfully on MMN (Modified Melin-Norkrans) medium. Desert truffle mycelium can be used directly from the plates as inoculum for in vitro mycorrhizal synthesis (Morte et al. 1994; Morte and Honrubia 1995, 1997) and from liquid fermentation for both in vitro and in vivo inoculation trials (Morte et al. 2008). However, only fungal strains well adapted to in vitro conditions should be used to produce mycelium by liquid fermentation in a bioreactor. In this sense, we have obtained a mycelium biomass (grams of dry weight/liter medium) of 0.41 g/l for *Picoa lefebvrei* (Santiago-Marín 2015), 0.30 g/l for *Terfezia claveryi* (Arenas 2014), and 1.16 g/l for *Terfezia olbiensis* (Morte et al. 2004). Similar values have been obtained for other ectomycorrhizal fungi, like *Pisolithus microcarpus* (0.48 g/l) by Rossi et al. (2002). However, other ectomycorrhizal fungi presented more vigorous growth in a bioreactor than desert truffles, like *Lactarius quieticolor* (3.26 g/l) and *Rhizopogon roseolus* (8.5 g/l) (Chávez et al. 2014). Therefore, greater effort is needed in order to increase these mycelium biomasses to ensure the continuous production of mycorrhizal plants.

Fungi produce a wide variety of secondary metabolites. In a wide generalization, Hanson (2008) considered that extracellular metabolites isolated from the culture filtrate may be associated with the combative relationship of the organism with its environment, while those isolated from the mycelium may have a protective role. Some of these substances are able to inhibit the development of their own populations (Trinci and Whittaker 1968). Thus, the erratic growth of the mycelium of *T. claveryi* may be caused by compounds produced by the fungus itself. To check

Fig. 2.2 Effect of adding different types of cyclodextrins (CDs) to the culture medium on the mycelial growth (cm) of *T. claveryi*. (circle) control; (diamond) γ -CD; (square) α -CD; and (triangle) β -CD (From López-Nicolás et al. 2013)



this, we tested the effect of several cyclodextrins (CDs) on *T. claveryi* mycelium. CDs are non-reducing cyclic glucose oligosaccharides that are produced as a result of the transformation of starch by certain bacteria. Two characteristics of CDs, the existence of a hydrophobic cavity and the presence of two hydrophilic hydroxyl rings, allow them to form inclusion complexes in water with a variety of organic guest molecules, such as volatile compounds (López-Nicolás et al. 2009) and phenols. CDs are able to stimulate the mycelium growth of the desert truffle *T. claveryi*, increasing fourfold the values of colony diameter, growth rate, and colony fresh weight after cultivation (López-Nicolás et al. 2013). The increase in mycelium growth observed when CDs are added to the culture medium is probably due to the formation of an inclusion complex and not to the CDs being used as a carbon source. β -CD (8 mmol l^{-1}) was seen to be the most effective natural CD to stimulate the mycelium growth of the *T. claveryi* (Fig. 2.2). The inner diameter of the hydrophobic cavity of β -CD, a structure formed by seven molecules of glucose, leads to a more favorable interaction between the CDs and the different molecules present in the culture medium that would otherwise hinder the correct growth of this desert truffle (López-Nicolás et al. 2013).

2.3.3 Mycorrhizal Plant Production

For the production of desert truffle mycorrhizal plants, four in vivo and two in vitro inoculation options were designed, the time required for each of them ranging between 4 and 8.5 months, depending on the type of plant propagation system and inoculum source used (Fig. 2.3). The photoautotrophic *Helianthemum* micropropagation system allowed this time to be reduced to 12 weeks with respect to photomixotrophic system since fungal inoculation is carried out at the moment plants are transferred from in vitro to ex vitro conditions, so that

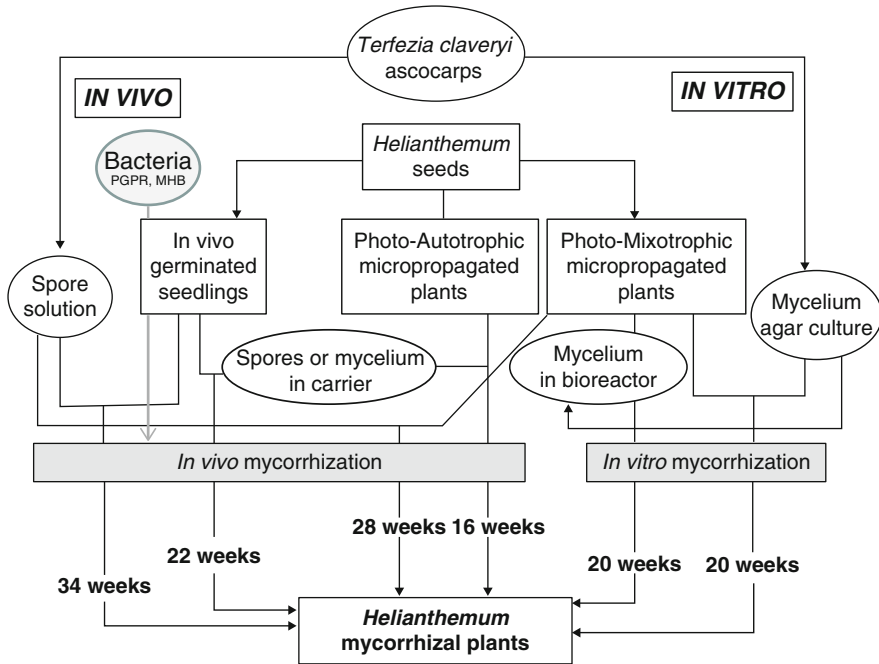


Fig. 2.3 In vivo and in vitro ways for production of desert truffle mycorrhizal plants and time required for each of them. *PGPR* plant growth-promoting rhizobacteria, *MHB* mycorrhiza helper bacteria

plant acclimatization and mycorrhization occur at the same time (Morte and Andrino 2014).

The last and most recent improvement to this protocol was the use of native bacteria (PGPR or MHB) in the mycorrhizal plant production system starting from *Helianthemum* seeds and desert truffle spores. This system is subdivided into three different stages: (i) seed germination, which includes seed germination itself and the development of true leaves for about 4 weeks; (ii) plant growth, which includes shoot elongation, plant hardening, and the development of secondary fine roots; and (iii) inoculation and mycorrhization, which includes shoot and root development and mycorrhization. The use of a combination of bacteria that includes four PGPR traits (Table 2.3), strain 5 (*Ps. fluorescens*) and strain 7 (*Pseudomonas* sp) at stage (i) and the strain 29 (*Ps. mandelii*) at stage (iii), increased seedling survival and growth (stage i) and mycorrhization percentage (stage iii), respectively. Our next objective will be the use of these bacteria in each one of the plant production routes to improve plant quality and reduce plant production time.

2.4 Advances in Understanding Desert Truffle Enzymes

The symbiosis between *T. claveryi* and *H. almeriense* is an ectendomycorrhiza (Gutiérrez et al. 2003; Navarro-Ródenas et al. 2012a) that is well adapted to drought conditions in poor soils. In order to understand these adaptations as well as the process of the fungal fruit-body formation, some enzymes involved in the primary and secondary metabolisms were studied during the different stages of the fungal life cycle.

Phosphatases have traditionally been classified as alkaline (ALP) or acid (ACP). Two peaks of activity were observed when phosphatase was measured in the crude extract of *Terfezia claveryi* ascocarps, one with maximum activity between pH 9.5 and 10.0, corresponding to ALP and another, of lower activity, with a maximum at pH 4.5 (Navarro-Ródenas et al. 2009). These results indicate that *T. claveryi* ascocarps contain both ACP and ALP, the latter being the main one. In addition to the optimum pH, ALP and ACP from *T. claveryi* ascocarps differ in their optimum temperature, around 45 °C for ALP and 35 °C for ACP. The thermostability of both enzymes at their respective optimum temperatures is also different: ALP activity decreases slightly with time at 45 °C, so that 25% of the initial activity is lost after 1 h (Navarro-Ródenas et al. 2009) while at 35 °C, in the same period, ACP loses 60% of its initial activity. This temperature sensitivity was one of the reasons that impaired ACP purification despite the various approaches assayed. A single ALP isoform was extracted and partially purified by precipitation with polyethylene glycol, a protocol that allowed elimination of ACP and most of the lipids and phenolic compounds. The gentle extraction method used, without sonication and with a buffer of high ionic strength and without the addition of detergent, indicates that the partially purified ALP is a soluble enzyme (Navarro-Ródenas et al. 2009).

The response of ACP and ALP to certain inhibitors also differed, which represents a useful tool for measuring each activity independently in a crude extract. Tartrate, a classical inhibitor of ACP, when present in the reaction medium at 1 mM, produced a 20% inhibition of *T. claveryi* ACP but had only a limited inhibitory effect (5.9%) on ALP (Navarro-Ródenas et al. 2009). At the same concentration, orthovanadate produced 70% inhibition in ALP and 80% inhibition of ACP. Kinetic analysis of the effect of orthovanadate confirmed that it is a competitive inhibitor of *T. claveryi* ALP.

Our group also reported the presence of ALP in *T. claveryi* mycelium, where it was seen to respond to water stress and could be used as an indicator of the metabolic activity present (Navarro-Ródenas et al. 2011, 2012a). The presence of ALP both in mycelia and ascocarps indicates that this enzyme must play an important role during the life cycle of *T. claveryi*, while ACP might be involved in a process that takes place during the ascocarp stage.

Two oxidoreductases (tyrosinase and lipoxygenase) were also isolated and characterized from *T. claveryi* ascocarps (Pérez-Gilabert et al. 2001a, b, 2005a, b).

Although the physiological role of most of these enzymes is not clear, their activity may affect the flavor, color, and texture of their ascocarps.

Tyrosinase (EC 1.14.18.1) is a copper-containing bifunctional monooxygenase, which uses molecular oxygen to catalyze the oxidation of monophenols to their corresponding *o*-diphenols (monophenolase or cresolase activity) (Pérez-Gilabert et al. 2001a) and their subsequent oxidation to *o*-quinones (diphenolase or catecholase activity) (Pérez-Gilabert et al. 2001b).

T. claveryi tyrosinase, extracted both from mature and immature ascocarps, is one of the few fully latent tyrosinases which have been characterized to date. However, activity could only be detected from the enzyme extracted from ascocarps if an activating agent such as SDS or trypsin was added to the reaction medium (Pérez-Gilabert et al. 2001a, b). The use of SDS as an activating agent is interesting since this detergent is known to inactivate many enzymes.

Both cresolase and catecholase activities of tyrosinase have been localized in the peridium, hypothecium, and the ascogenic hyphae of the gleba (Pérez-Gilabert et al. 2001a, b), which seem to be the most metabolically active tissues in the truffle ascocarp. This co-localization confirms the bifunctional character of this enzyme.

Lipoxygenases (LOXs) are non-heme iron-containing dioxygenases that catalyze the insertion of molecular oxygen into polyunsaturated fatty acids containing one or more 1,4 *Z,Z*-pentadiene systems, yielding the corresponding hydroperoxides. These hydroperoxides are subsequently metabolized *via* several secondary pathways giving rise to molecules, the so-called oxylipins, which have a wide range of biological functions (Brash 1999; Brodhun and Feussner 2011). LOXs are present in a wide variety of plant and animal tissues. Some plant LOXs are constitutive, whereas others are expressed by wounding and by fungal pathogens (Oliv 2002).

Due to the high proportion of polyunsaturated fatty acids present in *T. claveryi* ascocarps, lipid rancidity is one of the main factors limiting the storage life of this fungus, since lipid peroxidation gives rise to unpleasant odors and tastes, leading to consumer rejection. Enzymes such as LOX can accelerate the spoilage caused by oxidative rancidity. Hydroperoxides produced by this enzyme decompose to form volatile aroma compounds, which are perceived as off-flavors (Gordon 2001). In addition, the free radicals formed during lipid oxidation may also lead to a reduction in nutritional quality by reacting with vitamins, especially vitamin E, which is lost from foods during its action as antioxidant.

LOXs are classified according to the positional specificity of their products. Linoleic acid represents 45.4 % of total fatty acids in *T. claveryi* ascocarps, while linolenic acid represents 5.8% (Murcia et al. 2003). When the substrate specificity of the purified LOX was investigated, the highest relative activity was obtained using linoleic acid (100%), followed by linolenic acid (91%) and γ -linolenic acid (32%) (Pérez-Gilabert et al. 2005a). So, the specificity of purified LOX was characterized using linoleic and linolenic acid at the pH optimum of this enzyme (pH 7.0) and at pH 10.0, at which values a single peak corresponding to the 13-hydroperoxide was obtained with both substrates (Pérez-Gilabert et al. 2005a).

Thus, LOX from ascocarps can be considered a 13-LOX, similarly to the lipxygenases from *P. ostreatus* (Kuribayashi et al. 2002) and *Gäumannomyces graminis* (Su and Oliw 1998). Although there is little information on fungal LOX and its physiological role (Brodhun and Feussner 2011), the synthesis of a single specific hydroperoxide from free fatty acid substrates is related to the formation of biological mediators of signaling molecules (Brash 1999). Although several authors have studied the effect of plant oxylipins in arbuscular mycorrhiza (León Morcillo et al. 2012), the effect of fungal LOX on mycorrhizal symbiosis needs to be clarified.

2.5 Desert Truffle Plantations and Sylviculture

The cultivation of desert truffles is a very new commercial activity, with only 16 years of history. This cultivation is quite complicated by the species themselves and by the climatology of the cultivated areas, so it is a challenge both for basic and applied researchers to make the practice sustainable and profitable.

The first step in the establishment of a desert truffle plot is to choose suitable host plants and fungal species that are well adapted to the environmental conditions and soil characteristics. Moreover, high quality mycorrhizal plants, with certified mycorrhization levels, should be selected (Honrubia et al. 2014). All the cultivation practices necessary for desert truffle plantation management and sylviculture to improve wild production were well documented by Honrubia et al. (2014). Desert truffle fructification usually occurs 1–3 years after plantation, depending on mycorrhized seedling quality, site suitability, season and framework of the plantation, as well as management practices, mostly irrigation and weed elimination.

Numerous plantations have been established in Spain with the *Helianthemum* species *H. almeriense*, *H. violaceum*, *H. hirtum*, or *H. lipii* as host plants and *T. claveryi*, *Picoa lefebvrei* or *Tirmania nivea* as desert truffles (Fig. 2.4). Moreover, experimental results are available for the cultivation of *Terfezia boudieri* in Tunisia (Slama et al. 2010) and Israel (Kagan-Zur, pers.). All the species of *Terfezia* cultivated until now are typical of alkaline or basic soils. For the cultivation of acid soil species, we recently established, in April 2016, an experimental plot of 500 plants with the species of *Cistus salviifolius* and *Cistus ladanifer* mycorrhizal with *Terfezia arenaria*, a highly prized and widely consumed species. This plantation is located in the province of Cáceres (Extremadura, Spain), where we hope to apply our experience gained with species suited to alkaline soils.

There are two important factors that need to be taken into account to stimulate ascocarp production: a high density of mycorrhizal plants and adequate irrigation. As regard the first factor, a successful plantation frame was 1 × 1 m in rows separated by 2 m, which gave the first ascocarps after 2 years (Fig. 2.4). The small size of these shrubs allows to arrange them closer and thus optimizing the cultivated field. This means a plantation of around 8000 plants/ha, which, while very expensive to establish, could be amortized after 5 years of cultivation if production is

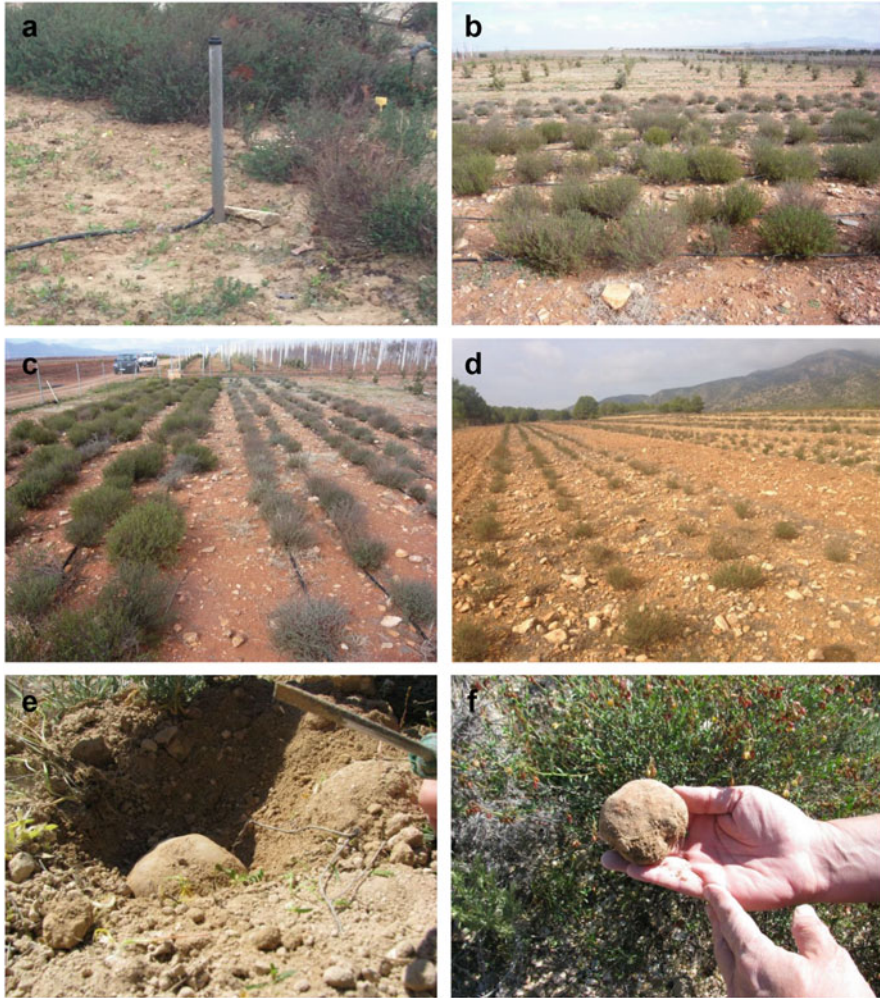


Fig. 2.4 Irrigation system can be provided by sprinklers (a) or drippers (b, c) in plantations of *H. almeriense* and *H. violaceum* with *T. claveryi* in Murcia (Spain) (a, b, c, d). Production of *T. claveryi* ascocaps in spring (e, f)

adequate (200–450 kg/ha). Adequate irrigation involves the amount of water needed and the time at which it is applied. After following *T. claveryi* production for 10 years in an orchard established in 1999, we observed a statistical correlation between the amount of precipitation during autumn (September, October, and November) and *T. claveryi* truffle production the following year (Morte et al. 2012). Therefore, it is essential to irrigate during these months if rainfall is not enough or does not occur. We estimated an irrigation of 50 l/ha/month in the region of Murcia according to the soil characteristics (loamy clay soil) in order to keep soil

matric potential at around -75 and -100 KPa. The irrigation system can use drippers or by sprinklers (Fig. 2.4).

In field plots in Murcia (Spain), drought stress significantly affected the mycorrhizal colonization percentage, which was 70% in non-irrigated mycorrhizal plants and 48% in irrigated mycorrhizal plants. However, no significant differences in plant growth were observed between non-irrigated and irrigated mycorrhizal plants before and after drought stress (Morte et al. 2010). Under drought stress, stomatal conductance was more sensitive to water stress than photosynthesis. There was a high degree of stomatal closure under water deficit and low radiation conditions, which improved water use efficiency in the plants grown under drought conditions (Morte et al. 2010).

The molecular base of this drought tolerance can be explained by the expression patterns of some aquaporin genes isolated from *H. almeriense* and from *T. claveryi* (Navarro-Ródenas et al. 2012b, 2013). Some of these aquaporins were subjected to fine-tuned expression only under drought-stress conditions. A beneficial effect on plant physiological parameters was observed in mycorrhizal plants compared with nonmycorrhizal ones. Moreover, stress induced a change in the mycorrhizal type formed, which was more intracellular under drought stress. The combination of a high rate of intracellular colonization and the fine-tuned expression of aquaporins could result in a morpho-physiological adaptation of this symbiosis to drought conditions (Navarro et al. 2013).

An alternative to desert truffle cultivation is to use suitable ecosystems in open forests managed in order to maintain and increase the productivity of these areas, in what is called *desert truffle sylviculture*, for which strategies were well defined in Honrubia et al. (2014). The sustainability of the desert truffle ecosystems implies a compromise between exploiting all the resources they harbor and respecting all the interests involved and those that may arise as social demands change. Only by producing (through the exploitation of resources), conserving (following criteria of sustainability), and improving (as regards the biodiversity and multifunctionality of the space in question) will ecosystem management offer guarantees for the future development of rural zones (Honrubia et al. 2014).

A successful example of this desert truffle sylviculture in natural production areas has been carried out in Abu Dhabi (UAE), where it was possible to stimulate the production of *T. boudieri* and *T. nivea* by sprinkler irrigation and spore inoculation of areas in the presence of *H. lippii* plants. In addition, the area was fenced in order to prevent the consumption of truffles by animals (Gouws et al. 2014).

However, more studies on mycorrhizal plant water relations and photosynthetic parameters are necessary if we want to control desert truffle fruiting in the face of global climate change. Future efforts of our group will be directed at deepening our knowledge of these subjects, so that, step by step, we will ultimately domesticate the cultivation of the desert truffle. The recent release of the sequencing and assembly for *Terfezia claveryi* genome, by the Joint Genome Institute and proposed by Dr. F Martin in collaboration with our group from the University of Murcia, may help to reveal the factors and enzymes required for the establishment and

maintenance of its interesting symbiosis, the formation of fruit bodies, and how climate change might affect the biology of this fungus.

Acknowledgements This work was supported by projects 19484/PI/14 (FEDER and Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia, Spain) and CGL2016-78946-R (AEI/FEDER, UE). JEMG thanks MINECO for a PhD grant (DI-14-06904). FA thanks MINECO for financial resources from the Youth Employment Initiative (JEI) and the European Social Fund (ESF), National System of Youth Guarantee (PEJ-2014-A-83659). ANR thanks the University of Murcia for a postdoctoral contract.

References

- Andrino A, Morte A, Honrubia M (2012) Method for producing plants of the *Cistaceae* family that establish mycorrhiza with different desert truffle species. Patent ES2386990, Spain
- Arenas F (2014) Optimización del crecimiento micelial y producción de inóculo de la trufa del desierto *Terfezia claveryi* Chatin en biorreactor. Máster Thesis, University of Murcia, Spain
- Bonifacio E, Morte A (2014) Soil properties. In: Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A (eds) Desert truffles, Soil biology, vol 38. Chapter 4. Springer, Berlin, pp 57–67. doi:10.1007/978-3-642-40096-4_4
- Bordallo JJ, Rodríguez A (2014) Cryptic and new species. In: Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A (eds) Desert truffles, Soil biology, vol 38. Chapter 3. Springer, Berlin, pp 39–53. doi:10.1007/978-3-642-40096-4_3
- Bordallo JJ, Rodríguez A, Honrubia M, Morte A (2012) *Terfezia canariensis* sp. nov. una nueva especie de trufa encontrada en las Islas Canarias. Cantarela 56:1–8
- Bordallo JJ, Rodríguez A, Muñoz-Mohedano JM, Suz LM, Honrubia M, Morte A (2013) Five new *Terfezia* species from the Iberian Peninsula. Mycotaxon 124:189–208
- Bordallo JJ, Rodríguez A, Kaounas V, Camello F, Honrubia MA (2015) Two new *Terfezia* species from Southern Europe. Phytotaxa 230:239–249
- Brash AR (1999) Lipoxigenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem 274:23679–23682
- Brodhun F, Feussner I (2011) Oxylipins in fungi. FEBS J 278:1047–1063
- Chávez D, Machuca A, Aguirre C, Palfner G (2014) Optimización del crecimiento micelial de los hongos ectomicorrízicos *Lactarius quieticolor* y *Rhizopogon roseolus* utilizando metodología de superficie de respuesta. XXII Congreso Latinoamericano de Microbiología-ALAM, Cartagena de Indias, Colombia, 4–8 noviembre 2014
- Gordon MH (2001) The development of oxidative rancidity in foods. In: Pokorny J, Yanishlieva N, Gordon M (eds) Antioxidants in food. Practical applications. CRC Press, Washington, DC, pp 7–20
- Gouws A, De Wet T, Abdullah F, Hassan A, Honrubia M, Morte A (2014) Desert truffle research in U.A.E. Abstract book of Second Symposium on Hypogeous Fungi in Mediterranean basin (HYPOGES2) & Fifth Congress Tuber aestivum/uncinatum European Scientific Group (TAUESG5), Université Mohammed V, Rabat (Morocco), 9–13 April 2014, p 17
- Gutiérrez A, Morte A, Honrubia M (2003) Morphological characterization of the mycorrhiza formed by *Helianthemum almeriense* Pau with *Terfezia claveryi* Chatin and *Picoa lefevrei* (Pat.) Maire. Mycorrhiza 13:299–307
- Hanson JR (2008) The chemistry of fungi. Royal Society of Chemistry, Cambridge
- Honrubia M, Gutiérrez A, Morte A (2001) Desert truffle plantation from south-east Spain. In: Edible mycorrhizal mushrooms and their cultivation. Proceedings of the second international conference on Edible Mycorrhizal Mushrooms, Christchurch, New Zealand, pp 3–5

- Honrubia M, Andrino A, Morte A (2014) Domestication: preparation and maintenance of plots. In: Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A (eds) Desert truffles. Soil biology, vol 38. Springer, Berlin, pp 367–387. Chapter 22. ISBN 978-3-642-40095-7. doi:[10.1007/978-3-642-40096-4_22](https://doi.org/10.1007/978-3-642-40096-4_22)
- Iotti M, Piattoni F, Leonardi P, Hall IR, Zambonelli A (2016) First evidence for truffle production from plants inoculated with mycelial pure cultures. *Mycorrhiza* 26:793–798. doi:[10.1007/s00572-016-0703-6](https://doi.org/10.1007/s00572-016-0703-6)
- Kagan-Zur V, Turgeman T, Roth-Bejerano N, Morte A, Sitrit Y (2014a) Benefits conferred to plants. In: Desert truffles. Soil biology, vol 38. Springer, Berlin, pp 93–104. doi:[10.1007/978-3-642-40096-4_7](https://doi.org/10.1007/978-3-642-40096-4_7)
- Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A (eds) (2014b) Desert truffles. Phylogeny, physiology, distribution and domestication, Soil biology, vol 38. Springer, Berlin. doi:[10.1007/978-3-642-40096-4](https://doi.org/10.1007/978-3-642-40096-4)
- Kovács G, Calonge D, Martín MP (2011) The diversity of *Terfezia* desert truffles: new species and a highly variable species complex with intrasporocarpic nrDNA ITS heterogeneity. *Mycologia* 103:841–853. doi:[10.3852/10-312](https://doi.org/10.3852/10-312)
- Kuribayashi T, Kaise H, Uno C, Hara T, Hayakawa T, John T (2002) Purification and characterization of lipoxygenase from *Pleurotus ostreatus*. *J Agric Food Chem* 50:1247–1253
- León Morcillo RJ, Ocampo JA, García Garrido JM (2012) Plant 9-lipoxygenase metabolism in response to arbuscular mycorrhiza. *Plant Signal Behav* 7:1584–1588
- López-Nicolas JM, Andreu-Sevilla AJ, Carbonell-Barrachina AA, García-Carmona F (2009) Effects of addition of alpha-cyclodextrin on the sensory quality, volatile compounds, and color parameters of fresh pear juice. *J Agric Food Chem* 57:9668–9675
- López-Nicolás JM, Pérez-Gilabert M, Lozano-Carrillo C, García-Carmona F, Morte A (2013) Mycelium growth stimulation of the desert truffle *Terfezia claveryi* Chatin by β -cyclodextrin. *Biotechnol Prog* 29:1558–1564
- Morte A, Andrino A (2014) Domestication: preparation of mycorrhizal seedlings. In: Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A (eds) Desert truffles, Soil biology, vol 38. Chapter 21. Springer, Berlin, pp 343–365. doi:[10.1007/978-3-642-40096-4_21](https://doi.org/10.1007/978-3-642-40096-4_21)
- Morte A, Honrubia M (1992) In vitro propagation of *Helianthemum almeriense* Pau (Cistaceae). *Agronomie* 12:807–809
- Morte A, Honrubia M (1995) Improvement of mycorrhizal synthesis between micropropagated *Helianthemum almeriense* plantlets with *Terfezia claveryi* (desert truffle). In: Elliot TJ (ed) Science and cultivation of edible fungi, vol 2. Balkema, Rotterdam, pp 863–868
- Morte A, Honrubia M (1997) Micropropagation of *Helianthemum almeriense*. In: Bajaj YPS (ed) High-tech and micropropagation VI, vol 40. Springer, Berlin. ISBN 3-540-61607-1
- Morte A, Cano A, Honrubia M, Torres P (1994) In vitro mycorrhization of micropropagated *Helianthemum almeriense* plantlets with *Terfezia claveryi* (desert truffle). *Agric Sci Finland* 3:309–314
- Morte A, Dieste C, Díaz G, Gutiérrez A, Navarro A, Honrubia M (2004) Production of *Terfezia olbiensis* mycelial inoculum in a bioreactor. Act 1er Symp Champignons Hypoges du Basin Méditerranéen, Rabat, Morocco, pp 146–149
- Morte A, Honrubia M, Gutiérrez A (2008) Biotechnology and cultivation of desert truffles. In: Varma A (ed) Mycorrhiza: state of the art genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics. Springer, Berlin, pp 467–483
- Morte A, Zamora M, Gutiérrez A, Honrubia M (2009) Desert truffle cultivation in semiarid Mediterranean areas. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (eds) Mycorrhizas – functional processes and ecological impact. Springer, Berlin, pp 221–233. doi:[10.1007/978-3-540-87978-7_15](https://doi.org/10.1007/978-3-540-87978-7_15)
- Morte A, Navarro-Ródenas A, Nicolás E (2010) Physiological parameters of desert truffle mycorrhizal *Helianthemum almeriense* plants cultivated in orchards under water deficit conditions. *Symbiosis* 52:133–139. doi:[10.1007/s13199-010-0080-4](https://doi.org/10.1007/s13199-010-0080-4)

- Morte A, Andrino A, Honrubia M, Navarro-Ródenas A (2012) *Terfezia* cultivation in arid and semiarid soils. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms, Soil biology, vol 34. Springer, Berlin. doi:[10.1007/978-3-642-33823-6_14](https://doi.org/10.1007/978-3-642-33823-6_14)
- Murcia MA, Martínez-Tomé M, Jimémez AM, Vera AM, Honrubia M, Parras P (2002) Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. *J Food Prot* 65:1614–1622
- Murcia MA, Martínez-Tomé M, Vera A, Morte A, Gutiérrez A, Honrubia M, Jimémez AM (2003) Effect of industrial processing on desert truffles *Terfezia claveryi* Chatin and *Picoa juniperi* Vittadini: proximate composition and fatty acids. *J Sci Food Agric* 83:535–541
- Navarro-Ródenas A, Morte A, Pérez-Gilabert M (2009) Partial purification, characterization and histochemical localization of alkaline phosphatase from ascocarps of the edible desert truffle *Terfezia claveryi* Chatin. *Plant Biol* 11:678–685. doi:[10.1111/j.1438-8677.2008.00172.x](https://doi.org/10.1111/j.1438-8677.2008.00172.x)
- Navarro-Ródenas A, Lozano-Carrillo MC, Pérez-Gilabert M, Morte A (2011) Effect of water stress on *in vitro* mycelium cultures of two mycorrhizal desert truffles. *Mycorrhiza* 21:247–253. doi:[10.1007/s00572-010-0329-z](https://doi.org/10.1007/s00572-010-0329-z)
- Navarro-Ródenas A, Morte A, Torrente P, Morte A (2012a) The role of phosphorus in the *ectendomycorrhiza continuum* of desert truffle mycorrhizal plants. *Mycorrhiza* 22:565–575. doi:[10.1007/s00572-012-0434-2](https://doi.org/10.1007/s00572-012-0434-2)
- Navarro-Ródenas A, Ruiz-Lozano JM, Kaldenhoff R, Morte A (2012b) The aquaporin TcAQP1 of the desert truffle *Terfezia claveryi* is a membrane pore for water and CO₂ transport. *Mol Plant Microbe Interact* 25:259–266
- Navarro-Ródenas A, Bárzana G, Nicolás E, Carra A, Schubert A, Morte A (2013) Expression analysis of aquaporins from desert truffle mycorrhizal symbiosis reveals a fine-tuned regulation under drought. *Mol Plant Microbe Interact* 26:1068–1078
- Navarro-Ródenas A, Berná LM, Lozano-Carrillo C, Andrino A, Morte A (2016) Beneficial native bacteria improve survival and mycorrhization of desert truffle mycorrhizal plants in nursery conditions. *Mycorrhiza* 26:769–779
- Oliw EH (2002) Plant and fungal lipoyxygenases. Prostaglandins Other Lipid Mediat 68–69:313–323
- Pérez-Gilabert M, Morte A, Honrubia M, García-Carmona F (2001a) Monophenolase activity of latent *Terfezia claveryi* tyrosinase: characterization and histochemical localization. *Physiologia Plantarum* 113:203–209
- Pérez-Gilabert M, Morte A, Honrubia M, García-Carmona F (2001b) Partial purification, characterization, and histochemical localization of fully latent desert truffle (*Terfezia claveryi* Chatin) polyphenol oxidase. *J Agric Food Chem* 49:1922–1927
- Pérez-Gilabert M, Sánchez-Felipe I, García-Carmona F (2005a) Purification and partial characterization of lipoyxygenase from desert truffle (*Terfezia claveryi* Chatin) ascocarps. *J Agric Food Chem* 53:3666–3671
- Pérez-Gilabert M, Sánchez-Felipe I, Morte A, García-Carmona F (2005b) Kinetic properties of lipoyxygenase from desert truffle (*Terfezia claveryi* Chatin) ascocarps: effect of inhibitors and activators. *J Agric Food Chem* 53:6140–6145
- Pérez-Gilabert M, García-Carmona F, Morte A (2014) Enzymes in *Terfezia claveryi* ascocarps. In: Desert truffles, Phylogeny, physiology, distribution and domestication, vol 38. Chapter 16. Springer, Berlin, pp 243–260. doi:[10.1007/978-3-642-40096-4_16](https://doi.org/10.1007/978-3-642-40096-4_16)
- Rossi MJ, Souza JAR, Oliveira VL (2002) Inoculum production of the ectomycorrhizal fungus *Pisolithus microcarpus* in an airlift bioreactor. *Appl Microbiol Biotechnol* 59:175–181
- Roth-Bejerano N, Navarro-Ródenas A, Gutiérrez A (2014) Types of mycorrhizal associations. In: Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A (eds) Desert truffles. Soil biology, vol 38. Springer, Berlin, pp 69–80. Chapter 5. ISBN 978-3-642-40095-7. doi:[10.1007/978-3-642-40096-4_5](https://doi.org/10.1007/978-3-642-40096-4_5)
- Santiago-Marín MM (2015) Producción de micelio de *Picoa lefebvrei* (Pat.) Maire en biorreactor. Máster Thesis, University of Murcia, Spain

- Shavit E (2014) The history of desert truffle use. In: Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A (eds) Desert truffles. Springer, Berlin, pp 217–242. doi:[10.1007/978-3-642-40096-4_15](https://doi.org/10.1007/978-3-642-40096-4_15)
- Slama A, Fortas Z, Boudabous A, Neffati M (2010) Cultivation of an edible desert truffle (*Terfezia boudieri* Chatin). Afr J Microbiol Res 4:2350–2356
- Su C, Oliw EH (1998) Manganese lipoxygenase, purification, and characterization. J Biol Chem 273:13072–13079
- Trinci AFJ, Whittaker C (1968) Self-inhibition of spore germination in *Aspergillus nidulans*. Trans Br Mycol Soc 51:594–596
- Zambonelli A, Donnini D, Rana GL, Fascetti S, Benucci GMN, Iotti M, Morte A, Khabar L, Bawadekji A, Piattoni F, Compagno R, Venturella G (2014) Hypogeous fungi in Mediterranean maquis, arid and semi-arid forests. Plant Biosyst 148:392–401

Chapter 3

The Role of Arbuscular Mycorrhizal Fungi and the Mycorrhizal-Like Fungus *Piriformospora indica* in Biocontrol of Plant Parasitic Nematodes

Ruchika Bajaj, Ram Prasad, Ajit Varma, and Kathryn E. Bushley

Abstract Fungal root symbionts have long been known to provide benefits to their plant hosts in terms of nutrient acquisition and growth promotion. The arbuscular mycorrhizal fungi (AMF) are ubiquitous symbionts of plants that help procure nutrients and protect plants from both abiotic and biotic stresses, including plant parasitic nematodes. Recently, the discovery of another group of mycorrhizal-like fungi belonging to the basidiomycete order Sebaciales have also been shown to colonize roots and assist their hosts in acquisition of nutrients as well as providing protection from a wide variety of both abiotic (drought, salinity, and temperature) and biotic (microbes, insects, and nematodes) stresses. *Piriformospora indica* is one such beneficial root symbiont discovered from the Thar Desert of Western India. It had been shown to enhance uptake of nutrients such as nitrogen, phosphorous, and potassium as well as some micronutrients and to alter plant hormones to promote plant growth and defense. It also recently has been shown to antagonize nematode growth and development. These fungi offer promise for the biocontrol of plant parasitic nematodes.

R. Bajaj

Department of Plant Biology, University of Minnesota, 822 BioSci Bldg, 1445 Gortner Avenue, St. Paul, MN 55108, USA

Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3 4th Floor, Sector 125, Noida 201303, Uttar Pradesh, India

R. Prasad • A. Varma

Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3 4th Floor, Sector 125, Noida 201303, Uttar Pradesh, India

K.E. Bushley (✉)

Department of Plant Biology, University of Minnesota, 822 BioSci Bldg, 1445 Gortner Avenue, St. Paul, MN 55108, USA

e-mail: kbushley@umn.edu

3.1 Introduction

Mycorrhiza is a combination of two classical Greek words, “mushroom” and “root.” Mycorrhiza represents a symbiotic association of the underground mycelia of fungi with plant roots without harming the plant. Mycorrhizal fungi are responsible in improving growth of host plant species due to increased nutrient uptake, production of growth promoting substances, and tolerance to biotic and abiotic stresses (Sreenivasa and Bagyaraj 1989). The Arbuscular Mycorrhizal Fungi (AMF) are widely distributed in natural and agricultural environments and have been found associated with more than 80% of land plants, liverworts, ferns, woody gymnosperms and angiosperms, and grasses (Smith and Read 2008). Recently, Basidiomycete fungi belonging to the order Sebaciales, including *Piriformospora indica* as well as *Sebacina* spp., have been shown to colonize the roots of a variety of agricultural crops and to provide similar benefits to plants in terms of growth promotion, nutrient acquisition, and protection from abiotic and biotic stress (Varma et al. 2012; Gill et al. 2016).

Plant parasitic nematodes (PPNs) represent one of the largest sources of uncontrollable biotic stress experienced by plants, causing as much as US\$173 billion in annual losses of crops worldwide (Elling 2013). They influence nearly all crops to some degree. The majority of crop damage is caused by the tylenchid nematodes, root-knot nematodes (RKN), and cyst nematodes (Bird 2004). The most damaging nematodes have sedentary endoparasitic lifestyles (Hussey and Roncadorl 1982; Vercauteren et al. 2002). The two main sedentary nematodes are the cyst nematodes (*Heterodera* and *Globodera*) and the root-knot nematodes (*Meloidogyne*) (Baum et al. 2007). In sedentary nematodes, the J2 larval worm stage invades the plant near the tip of a root and infects through the epidermal and cortex tissue and migrates to the developing vascular cells. The J2 nematodes inject their secretions into and around the plant cells to form the large feeder cell(s) (Caillaud et al. 2008). The feeding cells of cyst nematodes merge through the breakdown of neighboring cell walls to form the feeding structure known as the syncytium, through which the nematodes feed throughout their development (Ali et al. 2015). Feeding cells of root-knot nematodes (giant cells) form by repeated nuclear division in the absence of cell division (Abad et al. 2003). On the formation of feeding cells the juvenile nematode rapidly becomes sedentary because of their somatic muscles atrophy. The juveniles feed, enlarge, and molt three times to the adult stage. The large feeding cells formed by these nematodes plug the vascular tissue of the plant increasing susceptibility to water stress (Grundler and Hofmann 2011). Female sedentary endoparasites enlarge considerably into a saclike shape and are capable of laying large numbers of eggs. They are typically laid outside the nematode in a gelatinous egg mass, but in cyst nematodes most eggs are retained inside the female body which becomes melanized to encase and protect the eggs. Both types of nematodes have the same basic feeding strategy, but many cyst nematodes have an obligate sexual cycle (Cotton et al. 2014), whereas common species of RKN can reproduce largely by parthenogenesis (Ritz and Trudgill 1999).

3.2 Control of Plant Parasitic Nematodes

One of the main methods for control of PPN has been the use of resistant crop varieties. However, known resistance alleles are limited, breeding resistant varieties require large time and resource investments, and many PPN have already evolved to overcome plant resistance. Other agricultural practices such as crop rotation and the use of organic amendments have also been employed with some success (Timper 2014). Nematicides were once widely used to control PPN, but these chemicals are often associated with harmful environmental and health effects. For example, methyl bromide, one of the most important chemical fumigants used to control nematodes and other pests, affects a wide range of organisms, including beneficial microorganisms and humans, and is a chemical that contributes to the depletion of the Earth's ozone layer (Carpenter et al. 2001). In recent decades, concerns about the environmental and health hazards of using chemical nematicides and limited availability and durability of resistant crop varieties have led to increased interest in development of biological control agents, including fungi, as a component of crop protection (Grosch et al. 2005). Root symbionts such as AMF can compete with plant pathogens for nutrients and space by producing antibiotics, by directly parasitizing pathogens, or by inducing resistance in the host plants (Schouteden et al. 2015). Thus, these microbes have great potential for the biocontrol of nematodes and other soil-borne pathogens (Berg et al. 2007).

3.3 Role of AMF in Biocontrol of Nematodes

The biocontrol effect of AMF on soil-borne pathogens has been observed in a wide range of plant species and against many pathogens, many of them soil-borne fungi causing root rot or wilting (Azcon Aguilar and Barea 1996; Harrier and Watson 2004). However, they have also shown potential against both necrotrophic and biotrophic aboveground pathogens (Fritz et al. 2006) as well as nematode pests (Veresoglou and Rillig 2012; Schouteden et al. 2015). AMF have been shown to control PPN in a variety of temperate agricultural crop plants (Pinochet et al. 1996) such as tomato and carrot (Sousa et al. 2010), soybean (Oyekanmi et al. 2007), as well as tropical crops such as banana (Hol and Cook 2005). Although there are many research reports on the biocontrol effect of AMF, their actual use as biological control agents in the field is still not a routine agricultural practice (Salvioli and Bonfante 2013). This is partially due to variability in performance, depending on the AMF isolate, pathogen, plant species, and environmental conditions (Dong and Zhang 2006; Veresoglou and Rillig 2012; Salvioli and Bonfante 2013; Bajaj et al. 2017).

3.4 Mechanism of Biocontrol of Nematodes by AMF

The potential modes of action of AMF against nematodes include direct effects of AMF on the pathogen such as competition for space or nutrients or inhibition or indirect plant-mediated responses. The latter includes enhanced or altered plant growth, morphology and/or nutrition, biochemical changes associated with plant defense mechanisms, and changes in plant root exudates that promote antagonistic microbiota that leads to increased tolerance to nematodes (Whipps 2004; Schouteden et al. 2015). However, it has been observed that a threshold level of AMF colonization is a pre-requisite for many these plant responses (Cordier et al. 1998; Slezack et al. 2000). AMF also have the ability to induce systemic resistance against plant parasitic nematodes in roots (Elsen et al. 2008). The different mechanisms cannot be considered as completely independent from each other, and biocontrol probably results from a combination of these mechanisms (Vierheilig et al. 2008; Cameron et al. 2013). In addition, the relative importance of a specific mechanism can vary depending on the specific AMF–pathogen–plant interaction.

3.5 Increased Nutrient Uptake

The mutualistic relationship of AMF with plants increases the uptake of water and mineral nutrients, such as P, N, Ca, Cu, Mn, S, Zn, and Fe (Parniske 2008; Bajaj et al. 2014; Balliu et al. 2015), and in exchange the fungus receives photosynthetic carbon for their survival from their host (Gianinazzi et al. 2010). AMF protect the plant from both biotic and abiotic stresses (Chadha et al. 2015; Bajaj et al. 2015). Nematode damaged plants frequently show impaired water uptake through roots and deficiencies of N, B, Fe, Mg, and Zn, particularly. Cotton fields with better nutrient status were able to tolerate higher populations of when infested with *Rotylenchulus reniformis*, the sedentary semi-endoparasitic nematode, in their roots (Pettigrew et al. 2005). Cotton plants which were colonized with AMF, also showed increased Zn uptake, which contributed to tolerance against *Meloidogyne incognita* by reducing the detrimental nutrient deficiency imposed by RKN (Kantharaju et al. 2005). Regression analysis of nematode population densities against the mineral content in rice also revealed a positive correlation between the migratory ectoparasitic *Helicotylenchus* spp. and Mg. However, a negative correlation was observed between the migratory endoparasitic nematode *Pratylenchus zae* and Zn or Fe, and between *Meloidogyne incognita* and Mg and Ca (Coyne et al. 2004). These observations indicate that the nutrient status of the host plant can affect PPN population densities in both positive and negative ways.

3.6 Altered Root Morphology

In addition to increased nutrient uptake, mycorrhiza-colonized plants have enhanced root growth and branching (Gamalero et al. 2010; Gutjahr and Paszkowski 2013). Increased root growth may help the plant to counterbalance suppression of root growth caused by PPN. For example, this ability of AMF was observed in the banana tree where decreased root branching caused by the migratory endoparasitic nematodes was overcome by colonization with the Glomeromycete *Funneliformis mosseae* (Elsen et al. 2003).

3.7 Competition for Nutrients and Space

The PPN and fungi share similar physiological requirements and ecological niches. Thus, there can be competition for nutrients and space between these two groups of organisms, especially when critical nutrient sources such as carbon are limited (Vos et al. 2014). Several studies have demonstrated nutrient competition between AMF and fungal pathogens with respect to carbon (Hammer et al. 2011; Vos et al. 2014), but there is not much evidence for direct competition with nematodes (Jung et al. 2012). Similarly, since AMF and PPN both reside in and derive their nutrition from roots, they may also compete for space (Jung et al. 2012). The suppression of growth by PPN could be because the arbuscules of mycorrhiza are formed in the cortex, the same region where migratory PPN feed. This is not the case for cyst nematodes which feed on syncytia, with the feeding cells confined within the endodermis and thus less affected by AMF (Schouteden et al. 2015).

3.8 AMF-Induced Systemic Resistance

Systemic biological control of several pathogens has been reported to result from indirect effects resulting from changes in the host plant (Shoresh et al. 2010; Vos et al. 2012a; Song et al. 2011). Recently, it has been reported that the induction of systemic plant defense responses by AMF occurs because MAMP (microbe-associated molecular patterns) are conserved between beneficial and pathogenic fungi (Zamioudis and Pieterse 2012). Thus, AMF may be considered as putative pathogens by plants (Paszkowski 2006). When the plant's pattern recognition receptors recognize MAMP, a MAMP-triggered immunity response (MTI) is activated which forms the first line of defense of the plant, inhibiting invasion of other pathogens (Jones and Dangl 2006). The systemic nature of the mycorrhiza-induced resistance was observed in banana colonized by *G. intraradices* against the migratory burrowing nematode *R. similis* (Elsen et al. 2008). On other hand, in

Ammophila arenaria, no systemic resistance against *P. penetrans*, a lesion nematode, was observed after colonization by native AMF (De la Peña et al. 2006).

3.9 Altered Roots Exudates

The symbiosis of plants with AMF often changes the biochemical composition and level of production of roots exudates. This, in turn, impacts the hatching, mobility, chemotaxis, and host localization by nematode juveniles (Vos et al. 2012a, b). Changes in root exudates could involve compounds such as sugars and organic acids (Hage-Ahmed et al. 2013), amino acids (Harrier and Watson 2004), flavonoids and strigolactones (Steinkellner et al. 2007), plant hormones, and phenolics (McArthur and Knowles 1992). There is ample evidence that root exudates can alter the rhizosphere microbiome (Lakshmanan et al. 2014). While few studies address this topic, it is possible that root exudates induced by AMF and other root symbionts promote rhizosphere communities antagonistic to nematodes (Vos et al. 2012a, b). The level of colonization and the particular symbiont involved also impacts root exudates in the rhizosphere (Kobra et al. 2009; Lioussanne et al. 2008), and it is believed that a threshold level of colonization is also required for this mechanism of biocontrol (Paulitz 2000; Chatterton and Punja 2011).

3.10 Role of *Piriformospora indica* in Biocontrol of Nematodes

Piriformospora indica, a Basidiomycetes of the order Sebaciniales, is an endophytic symbiotic fungus which was isolated from rhizosphere of the xerophytic woody shrubs from the Thar deserts of Rajasthan, India (Verma et al. 1998; Varma et al. 2013, 2014). It has plant growth promotional activity while providing benefits of biotic and abiotic stress tolerance to the host plant (Gill et al. 2016). It also protects the plants from pathogens and herbivores (Verma et al. 1998; Deshmukh et al. 2006; Daneshkhah et al. 2013; Bajaj et al. 2015). Like AMF, it has an extensive range of hosts, colonizing members of the bryophytes, pteridophytes, gymnosperms, angiosperms (both monocots and dicots), and orchids. In the majority of plant species investigated, there are two distinct phases in the colonization of plants by *P. indica*. In initial stages of infection, *P. indica* acts as a biotroph, but later on acts as a necrotrophic, killing some cells of the plant root through apoptosis (Zuccaro et al. 2011) and essentially forming a saprophytic association (Deshmukh et al. 2006) However, in orchids, the fungus forms a symbiotic association with the plant that promotes root growth (Ye et al. 2014). *Piriformospora indica* increases nutrient uptake, particularly of phosphorus (Singh et al. 2000; Malla et al. 2004), and improves plant growth and stress tolerance by inducing phytohormones (Gill

et al. 2016; Siddhanta et al. 2017). Studies have revealed that it also can enhance the production of plant secondary metabolites (Bagde et al. 2010, 2014; Das et al. 2012, 2013; Kumar et al. 2012; Prasad et al. 2008, 2013; Bajaj et al. 2014).

3.11 Biotic Stress Tolerance

P. indica-infested plants are more resistant to biotic stresses. In barley infected with macroconidia of the necrotrophic fungal pathogen *Fusarium culmorum*, *P. indica*-infested plants were more tolerant to the devastating effect of *F. culmorum* root disease (Harrach et al. 2013). Root and shoot fresh weights were reduced only twofold in *P. indica*-colonized plants, compared with the 12-fold in controls with *F. culmorum* alone. Similar results were observed for the root pathogen *Crocus sativus*, which shows a hemibiotrophic nourishment strategy (Waller et al. 2005). These results show that *P. indica* exerts beneficial activity against major crop pathogens that cause enormous worldwide economic losses. Deshmukh et al. (2006) reported comparable biological activities of the treatments in terms of biomass increase and protection against biotrophic stress of *Blumeria graminis*, powdery mildew fungus in barley. Colonization of barley roots with *P. indica* induced systemic resistance against the biotrophic leaf pathogen. Analysis of a number of *Arabidopsis* mutants showed that jasmonate signaling is important for *P. indica*-induced resistance (Stein et al. 2008). A subset of defense-related genes are expressed earlier and more strongly induced by leaf pathogens in root endophyte-colonized barley plants than in control plants (Molitor et al. 2011). Hence, the mechanisms of *P. indica*-induced resistance seem to be similar to the well-characterized induced systemic resistance described for plant growth-promoting rhizobacteria-colonized plants (van Wees et al. 2008).

3.12 Biocontrol of Nematodes by *P. indica*

Daneshkhah et al. (2013) reported that colonization of *P. indica* on *Arabidopsis* roots in vitro antagonized the infection and development of cyst nematodes. In other fungi, this antagonistic activity can be elucidated by production of secondary fungal metabolites and enzymes such as chitinases that feature toxicity against parasitic nematodes (Shinya et al. 2008). Endophytic fungi are able to produce large amounts of toxic chemicals in vitro (Vu 2005), some of which may have direct nematocidal activity. Further studies are needed to determine if *P. indica* produces compounds with direct toxicity to nematodes, although its genome sequence showed few genes with known functions in fungal secondary metabolism (Zuccaro et al. 2011). Daneshkhah et al. (2013), however, noted that cell-wall extracts of *P. indica* alone significantly decreased nematode infection and development. *P. indica* may also impact production of plant secondary metabolites that deter

nematodes. *P. indica* root colonization affected J₂ infection, especially during the biotrophic phase. In this phase, the expression of *MYB51*, a plant gene involved in the biosynthesis of antimicrobial indole glucosinolates (Clay et al. 2009), is induced in roots of *P. indica*-treated plants (Jacobs et al. 2011). In roots inoculated with *P. indica*, it was observed that expression of *CBP60g* and *SID2*, markers of the salicylic acid-mediated signaling pathway, were upregulated (Jacobs et al. 2011). Therefore, salicylic acid-mediated signaling may also be involved in significant inhibitory effect on *H. schachtii*, since salicylic acid was revealed to inhibit growth of *H. schachtii* (Wubben et al. 2008).

3.13 Case Study: *Piriformospora indica* Antagonizes the Soybean Cyst Nematode in Planta

Field soil was collected from an agricultural field with no soybean cyst nematode infestation, mixed with 30% sand and autoclaved twice. Mycelium of *P. indica* at concentrations of 0% (w/w), 2.5% (w/w), and 5% (w/w) was thoroughly mixed with soil and placed into clay pots in a controlled greenhouse trial in order to analyze its possible effects on growth, development, and pest resistance towards the SCN (Bajaj et al. 2015, 2017).

Root colonization was observed by staining the roots with lactophenol cotton blue, and intracellular chlamydospores of *P. indica* were observed confined to the root cortex. Levels of root colonization ranged from 45% to 50% in 2.5% and 5% *P. indica* treatments at 8 weeks after planting. No colonization was observed in 0% *P. indica* treatment. Soybean showed a positive interaction with *P. indica*, as demonstrated by increased shoot biomass and shoot length of inoculated plants as compared to control plants. However, the overall biomass of colonized roots was lower than that of the uncolonized control roots. *P. indica* not only induces development of the vegetative aerial part of the plant, but also is responsible for early maturation with respect to flowering in soybean (Table 3.1).

The number of SCN eggs per cc soil, a common screening measure of SCN severity in agricultural fields, was significantly lower in the *P. indica* amended pots. There was a decrease of 29.7% in the 2.5% *P. indica* treatment and 36.7% in the 5% *P. indica* treatment. Egg density per cc soil was also significantly reduced between the 2.5% to the 5% *P. indica* treatments. Egg density calculated as number of eggs/cc soil/gram root wet or dry weight also showed a trend, although not significant, of decreasing egg density with increasing *P. indica* in soil.

Table 3.1 Effects of *P. indica* on soybean

<i>Piriformospora indica</i> colonization in <i>Glycine max</i>					
Increased flowering and pods	Decreased root volume	Decreased egg density of SCN	Increased nutrient uptake	Increased hormones	Increased metabolism in plants

Although the mechanism of nematode inhibition in this study is unknown, *P. indica* may either directly inhibit nematode development, as discussed above, or may control plant responses that impact nematode colonization and development. One possible plant response is altered carbon-partitioning in the plant. The fungus has been shown to control expression of a *Nicotiana* attenuate homolog of Hsl-Pro-1, a locus initially identified as having a role in resistance to the beet cyst nematode (*H. schachtii* Schmidt) (Cai et al. 1997), but now thought to be involved in more generalized responses to both abiotic and biotic stresses and in repartitioning of carbon resources within the plant (Schuck et al. 2012). Enzymes such as sucrose synthases and invertases may also impact development of cyst nematodes by altering plant sink strengths and changing systemic sugar partitioning to decrease syncytial sugar levels. Higher sugar levels in roots were shown to contribute to enhanced nematode development (Cabello et al. 2013) and have major nutritional value for this obligate parasite. Thus, allocation of sugars to shoot growth over root growth could impact the availability of sugars to root cyst nematodes. In contrast to AMF, *P. indica* decreased root growth and branching. However, decreased root growth may also reduce infection by the SCN (Bajaj et al. 2015) by lowering the number of potential sites for nematode infection (Schouteden et al. 2015).

3.14 Conclusions

AMF and other beneficial fungi such as *P. indica* confer many of the same benefits to plant hosts including improved nutrient uptake, increased plant growth, enhanced abiotic and biotic stress tolerance, induced systemic resistance against pathogens, and production or induction of plant protective secondary compounds or root exudates. However, there may also be significant differences in the mechanisms of nematode antagonism by these two groups of fungi. Instead of increasing root growth and proliferation like AMF, *P. indica* causes cell death in roots and directs resources to shoot growth, leading to smaller root:shoot ratios. Reduced root growth and volume may promote biocontrol of nematodes both by diverting sugars on which juvenile nematodes feed from root to shoot and also by providing less root surface area for infection. While further studies are needed to investigate nematode inhibitory compounds produced by AMF, to our knowledge, the majority of known mechanisms of AMF protection against nematodes are plant mediated. In contrast, compounds and cell-wall components of *P. indica* have been shown to directly inhibit infection and development of nematodes in roots. Both groups of fungi offer promising avenues for successful biocontrol of PPN.

Acknowledgment Ajit varma is thankful to DBT for partial funding and to DST for providing Confocal Microscope.

References

- Abad P, Favery B, Rosso MN, Castagnone-Sereno P (2003) Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Mol Plant Pathol* 4:217–224
- Ali S, Magne M, Chen S, Côté O, Stare BG, Obradovic N, Jamshaid L, Wang X, Bélair G, Moffett P (2015) Analysis of putative apoplast effectors from the nematode, *Globodera rostochiensis*, and identification of an expansin-like protein that can induce and suppress host defenses. *PLoS One* 10:e0115042
- Azcon Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. *Mycorrhiza* 6:457–464
- Bagde US, Prasad R, Varma A (2010) Interaction of *Piriformospora indica* with medicinal plants and of economic importance. *Afr J Biotechnol* 9:9214–9226
- Bagde US, Prasad R, Varma A (2014) Impact of culture filtrate of *Piriformospora indica* on biomass and biosynthesis of active ingredient aristolochic acid in *Aristolochia elegans* mart. *Int J Biol* 1:29–37
- Bajaj R, Agarwal A, Rajpal K, Asthana S, Kumar R, Prasad R, Kharkwal AC, Sherameti I, Oelmüller R, Varma A (2014) Co-cultivation of *Curcuma longa* with *Piriformospora indica* enhances the yield and active ingredients. *Am J Curr Microbiol* 2:6–17
- Bajaj R, Hu W, Huang Y, Chen S, Prasad R, Varma A, Bushley KE (2015) The beneficial root endophyte *Piriformospora indica* reduces egg density of the soybean cyst nematode. *Biol Control* 90:193–199
- Bajaj R, Chen S, Hu W, Huang Y, Prasad R, Kumar V, Tuteja N, Varma A, Bushley K (2017) Protocol for biocontrol of soybean cyst nematode with root endophytic fungi. In: Varma A, Sharma AK (eds) *Modern Tools and Techniques to Understand Microbes*. Springer, Cham
- Balliu A, Sallaku G, Rewald B (2015) AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. *Sustainability* 7:15967–15981
- Baum TJ, Hussey RS, Davis EL (2007) Root-knot and cyst nematode parasitism genes: the molecular basis of plant parasitism. In: Baum TJ, Hussey RS, Eric L (eds) *Genetic engineering*. Springer, New York, pp 17–43
- Berg G, Grosch R, Scherwinski K (2007) Risk assessment for microbial antagonists: are there effects on non-target organisms? *Gesunde Pflanzen* 59:107–117
- Bird DM (2004) Signaling between nematodes and plants. *Curr Opin Plant Biol* 7:372–376
- Cabello S, Lorenz C, Crespo S, Cabrera J, Ludwig R, Escobar C, Hofmann J (2013) Altered sucrose synthase and invertase expression affects the local and systemic sugar metabolism of nematode-infected *Arabidopsis thaliana* plants. *J Exp Bot* 65:201–212
- Cai DG, Kleine M, Kifle S, Harloff HJ, Sandal NN, Marcker KA, Klein Lankhorst RM, Salentijn EMJ, Lange W, Stiekema WJ, Wyss U, Grundler FMW, Jung C (1997) Positional cloning of a gene for nematode resistance in sugar beet. *Science* 275:832–834
- Caillaud MC, Dubreuil G, Quentin M, Perfus-Barbeoch L, Lecomte P, de Almeida EJ, Abad P, Rosso MN, Favery B (2008) Root-knot nematodes manipulate plant cell functions during a compatible interaction. *J Plant Physiol* 165:104–111
- Cameron D, Neal A, van Wees S, Ton J (2013) Mycorrhiza-induced resistance: more than the sum of its parts? *Trends Plant Sci* 18:539–545
- Carpenter J, Lynch L, Trout T (2001) Township limits on 1,3-D will impact adjustment to methyl bromide phase-out. *Calif Agric* 55:12–18
- Chadha N, Mishra M, Rajpal K, Bajaj R, Choudhary DK, Varma A (2015) An ecological role of fungal endophytes to ameliorate plants under biotic stress. *Arch Microbiol* 197:869–881
- Chatterton S, Punja ZK (2011) Colonization of geranium foliage by *Clonostachys rosea* f. *catenulata*, a biological control agent of botrytis grey mould. *Botany* 90:1–10
- Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM (2009) Glucosinolate metabolites required for an *Arabidopsis* innate immune response. *Science* 323:95–101

- Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. *Mol Plant Microbe Interact* 11:1017–1028
- Cotton JA, Lilley CJ, Jones LM, Kikuchi T, Reid AJ, Thorpe P, Tsai JJ, Beasley H, Blok V, Cock PJA, den Akker SE, Holroyd N, Hunt M, Mantelin S, Naghra H, Pain A, Palomares-Rius JE, Zarowiecki M, Berriman M, Jones JT, Urwin PE (2014) The genome and life-stage specific transcriptomes of *Globodera pallida* elucidate key aspects of plant parasitism by a cyst nematode. *Genome Biol* 15:R43. doi:10.1186/gb-2014-15-3-r43
- Coyne DL, Sahrawat KL, Plowright RA (2004) The influence of mineral fertilizer application and plant nutrition on plant-parasitic nematodes in upland and lowland rice in Côte d'Ivoire and its implications in long term agricultural research trials. *Exp Agric* 40:245–256
- Daneshkhal R, Cabello S, Rozanska E, Sobczak M, Grundler FMW, Wieczorek K, Hofmann J (2013) *Piriformospora indica* antagonizes cyst nematode infection and development in Arabidopsis roots. *J Exp Bot* 64:3763–3774
- Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri AK, Varma A (2012) The root endophyte fungus *Piriformospora indica* leads to early flowering higher biomass and altered secondary metabolites of the medicinal plant *Coleus forskohlii*. *Plant Signal Behav* 7:103–112
- Das A, Prasad R, Srivastava RB, Deshmukh S, Rai MK, Varma A (2013) Co-cultivation of plants with medicinal plants: case studies. In: Varma A, Kost G, Oelmüller R (eds) *Piriformospora indica*: Sebacinale and their biotechnological applications. Springer, Berlin, pp 149–171
- De la Peña E, Echeverría SR, Putten WH, Van Der Freitas H, Moens M (2006) Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytol* 169:829–840
- Deshmukh S, Hüchelhoven R, Schäfer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH (2006) The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. *Proc Natl Acad Sci* 103:18450–18457
- Dong LQ, Zhang KQ (2006) Microbial control of plant-parasitic nematodes: a five-party interaction. *Plant Soil* 288:31–45
- Elling AA (2013) Major emerging problems with minor *Meloidogyne* species. *Phytopathology* 103:1092–1102
- Elsen A, Beeterens R, Swennen R, De Waele D (2003) Effects of an arbuscular mycorrhizal fungus and two plant-parasitic nematodes on *Musa* genotypes differing in root morphology. *Biol Fertil Soils* 38:367–376
- Elsen A, Gervacio D, Swennen R, De Waele D (2008) AMF-induced biocontrol against plant-parasitic nematodes in *Musa* sp.: a systemic effect. *Mycorrhiza* 18:251–256
- Fritz M, Jakobsen I, Lyngkjær MF, Thordal-Christensen H, Pons-Kühnemann J (2006) Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. *Mycorrhiza* 16:413–419
- Gamalero E, Pivato B, Bona E, Copetta A, Avidano L, Lingua G, Berta G (2010) Interactions between a fluorescent pseudomonad, an arbuscular mycorrhizal fungus and a hypovirulent isolate of *Rhizoctonia solani* affect plant growth and root architecture of tomato plants. *Plant Biosyst* 144:582–591
- Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* 20:519–530
- Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. *Front Microbiol* 7:20. doi:10.3389/fmicb.2016.00332
- Grosch R, Lottmann J, Faltin F, Berg G (2005) Use of bacterial antagonists to control diseases caused by *Rhizoctonia solani*. *Gesunde Pflanzen* 57:199–205
- Grundler FM, Hofmann J (2011) Water and nutrient transport in nematode feeding sites. In: Jones J, Gheysen G, Fenoll C (eds) *Genomics and molecular genetics of plant-nematode interactions*. Springer, Dordrecht, pp 423–439

- Gutjahr C, Paszkowski U (2013) Multiple control levels of root system remodeling in arbuscular mycorrhizal symbiosis. *Front Plant Sci* 4:1–8
- Hage-Ahmed K, Moyses A, Voglgruber A, Hadacek F, Steinkellner S (2013) Alterations in root exudation of intercropped tomato mediated by the arbuscular mycorrhizal fungus *Glomus mosseae* and the soilborne pathogen *Fusarium oxysporum* f. sp. lycopersici. *J Phytopathol* 161:763–773
- Hammer EC, Pallon J, Wallander H, Olsson PA (2011) Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiol Ecol* 76:236–244
- Harrach BD, Baltruschat H, Barna B, Fodor J, Kogel KH (2013) The mutualistic fungus *Piriformospora indica* protects barley roots from a loss of antioxidant capacity caused by the necrotrophic pathogen *Fusarium culmorum*. *Mol Plant-Microbe Interact* 26:599–605
- Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. *Pest Manag Sci* 60:149–157
- Hol WHG, Cook R (2005) An overview of arbuscular mycorrhizal fungi-nematode interactions. *Basic Appl Ecol* 6:489–503
- Hussey RS, Roncadori RW (1982) Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Dis* 66:9–14
- Jacobs S, Zechmann B, Molitor A, Trujillo M, Petutschnig E, Lipka V, Kogel KH, Schäfer P (2011) Broad-spectrum suppression of innate immunity is required for colonization of *Arabidopsis* roots by the fungus *Piriformospora indica*. *Plant Physiol* 156:726–740
- Jones JDG, Dangl JL (2006) The plant immune system. *Nature* 444:323–329
- Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-induced resistance and priming of plant defenses. *J Chem Ecol* 38:651–664
- Kantharaju V, Krishnappa K, Ravichandra NG, Karuna K (2005) Management of root-knot nematode, *Meloidogyne incognita* on tomato by using indigenous isolates of AM fungus, *Glomus fasciculatum*. *Indian J Nematol* 35:32–36
- Kobra N, Jalil K, Youbert G (2009) Effects of three *Glomus* species as biocontrol agents against *Verticillium*-induced wilt in cotton. *J Plant Prot Res* 49:185–189
- Kumar V, Rajauria G, Sahai V, Bisaria VS (2012) Culture filtrate of root endophytic fungus *Piriformospora indica* promotes the growth and lignan production of *Linum album* hairy root cultures. *Process Biochem* 47:901–907
- Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an aboveground problem. *Plant Physiol* 166:689–700
- Lioussanne L, Jolicœur M, St-Arnaud M (2008) Mycorrhizal colonization with *Glomus intraradices* and development stage of transformed tomato roots significantly modify the chemotactic response of zoospores of the pathogen *Phytophthora nicotianae*. *Soil Biol Biochem* 40:2217–2224
- Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmueller R, Varma A (2004) Phosphorus solubilizing symbiotic fungus *Piriformospora indica*. *Endocytobiosis Cell Res* 15:579–600
- McArthur DAJ, Knowles NR (1992) Resistance responses of potato to vesicular-arbuscular mycorrhizal fungi under varying abiotic phosphorus levels. *Plant Physiol* 100:341–351
- Molitor A, Zajic D, Voll LM, Pons-Kühnemann J, Samans B, Kogel KH, Waller F (2011) Barley leaf transcriptome and metabolite analysis reveals new aspects of compatibility and *Piriformospora indica*-mediated systemic induced resistance to powdery mildew. *Mol Plant-Microbe Interact* 24:1427–1439
- Oyekanni EO, Coyne DL, Fagade OE, Osonubi O (2007) Improving root-knot nematode management on two soybean genotypes through the application of *Bradyrhizobium japonicum*, *Trichoderma pseudokoningii* and *Glomus mosseae* in full factorial combinations. *Crop Prot* 26:1006–1012
- Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* 6:763–775

- Paszkowski U (2006) Mutualism and parasitism: the yin and yang of plant symbioses. *Curr Opin Plant Biol* 9:364–370
- Paulitz TC (2000) Population dynamics of biocontrol agents and pathogens in soils and rhizospheres. *Eur J Plant Pathol* 106:401–413
- Pettigrew WT, Meredith WR, Young LD (2005) Potassium fertilization effects on cotton lint yield, yield components, and reniform nematode populations. *Agron J* 97:1245–1251
- Pinochet J, Calvet C, Camprubi A, Fernandez C (1996) Interactions between migratory endoparasitic nematodes and arbuscular mycorrhizal fungi in perennial crops: a review. *Plant Soil* 185:183–190
- Prasad R, Bagde US, Puspagadan P, Varma A (2008) *Bacopa monniera* L. pharmacological aspects and case studies involving *Piriformospora indica*. *Int J Integr Biol* 3:100–110
- Prasad R, Kamal S, Sharma PK, Oelmüller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. *J Basic Microbiol* 53:1016–1024
- Ritz K, Trudgill DL (1999) Utility of nematode community analysis as an integrated measure of the functional state of soils: perspectives and challenges. *Plant Soil* 212:1–11
- Salvioli A, Bonfante P (2013) Systems biology and “omics” tools: a cooperation for next-generation mycorrhizal studies. *Plant Sci* 203:107–114
- Schouteden N, De Waele D, Panis B, Vos CM (2015) Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: a review of the mechanisms involved. *Front Microbiol* 6. doi:10.3389/fmicb.2015.01280
- Schuck S, Camehl I, Gilardoni PA, Oelmüller R, Baldwin IT, Bonaventure G (2012) HSPRO controls early *Nicotiana attenuata* seedling growth during interaction with the fungus *Piriformospora indica*. *Plant Physiol* 160:929–943
- Shinya R, Aiuchi D, Kushida A, Tani M, Kuramochi K, Koike M (2008) Effects of fungal culture filtrates of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains on *Heterodera glycines* eggs and juveniles. *J Invertebr Pathol* 97:291–297
- Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu Rev Phytopathol* 48:21–43
- Siddhanta S, Paidi SK, Bushley K, Prasad R, Barman I (2017) Exploring morphological and biochemical linkages in fungal growth with label-free light sheet microscopy and Raman spectroscopy. *ChemPhysChem* 18:72–78. doi:10.1002/cphc.201601062
- Singh A, Sharma J, Rexer KH, Varma A (2000) Plant productivity determinants beyond minerals, water and light: *Piriformospora indica* – a revolutionary plant growth promoting fungus. *Curr Sci* 79:1548–1554
- Slezacek S, Dumas-Gaudot E, Paynot M, Gianinazzi S (2000) Is a fully established arbuscular mycorrhizal symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces eusteiches*. *Mol Plant Microbe Interact* 13:238–241
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic Press, London. ISBN-13: 9780123705266
- Song YY, Cao M, Xie LJ, Liang XT, Zeng RS, Su YJ, Huang JH, Wang RL, Luo SM (2011) Induction of DIMBOA accumulation and systemic defense responses as a mechanism of enhanced resistance of mycorrhizal corn (*Zea mays* L.) to sheath blight. *Mycorrhiza* 21:721–731
- Sousa CD, Soares ACF, Coimbra JL, Garrido MD, Machado GD (2010) Arbuscular mycorrhizal fungi in the control of *Meloidogyne incognita* in tomato seedlings. *Revista Caatinga* 23:15–20
- Sreenivasa MN, Bagyaraj DJ (1989) Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum. *Plant Soil* 119:127–132
- Stein E, Molitor A, Kogel KH, Waller F (2008) Systemic resistance in *Arabidopsis* conferred by the mycorrhizal fungus *Piriformospora indica* requires jasmonic acid signaling and the cytoplasmic function of NPR1. *Plant Cell Physiol* 49:1747–1751
- Steinkellner S, Lenzemo V, Langer I, Schweiger P, Khaosaad T, Toussaint JP, Vierheilig H (2007) Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant-fungus interactions. *Molecules* 12:1290–1306
- Timper P (2014) Conserving and enhancing biological control of nematodes. *J Nematol* 46:75–89

- van Wees SC, Van der Ent S, Pieterse CM (2008) Plant immune responses triggered by beneficial microbes. *Curr Opin Plant Biol* 11:443–448
- Varma A, Kharkwal AC, Bains KS, Agarwal A, Bajaj R, Prasad R (2012) *Piriformospora indica*: the model microbe for organic green revolution. *Org News*:1–6
- Varma A, Tripathi S, Prasad R, Das A, Sharma M, Bakshi M, Arora M, Rastogi K, Agrawal A, Kharkwal AC, Tsimilli-Michael M, Strasser RJ, Bagde US, Bisaria VS, Upadhyaya CP, Malla R, Kost G, Joy K, Sherameti I, Chen Y, Ma J, Lou B, Oelmüller R (2013) The symbiotic fungus *Piriformospora indica*: update. In: Hock B (ed) *The mycota IX*. Springer, Berlin, pp 21–254
- Varma A, Sree KS, Arora M, Bajaj R, Prasad R, Kharkwal AC (2014) Functions of novel symbiotic fungus – *Piriformospora indica*. *Proc Indian Natl Sci Acad* 80:429–441
- Vercauteren I, de Almeida EJ, De Groot R, Gheysen G (2002) An *Arabidopsis thaliana* pectin acetyltransferase gene is upregulated in nematode feeding sites induced by root-knot and cyst nematodes. *Mol Plant-Microbe Interact* 15:404–407
- Veresoglou SD, Rillig MC (2012) Suppression of fungal and nematode plant pathogens through arbuscular mycorrhizal fungi. *Biol Lett* 8:214–217
- Verma SA, Varma A, Rexer K-H, Hassel A, Kost G, Sarbhoy A, Bisen P, Bütehörn B, Franken P (1998) *Piriformospora indica* gen et sp nova new root-colonizing fungus. *Mycologia* 90:896–903
- Vierheilig H, Steinkellner S, Khaosaad T (2008) The biocontrol effect of mycorrhization on soil borne fungal pathogens and the autoregulation of the AM symbiosis: one mechanism, two effects? In: Varma A (ed) *Mycorrhiza*. Springer, Berlin, pp 307–320
- Vos C, Claerhout S, Mkwandwire R, Panis B, De Waele D, Elsen A (2012a) Arbuscular mycorrhizal fungi reduce root-knot nematode penetration through altered root exudation of their host. *Plant Soil* 354:335–345
- Vos C, Van den Broucke D, Lombi FM, De Waele D, Elsen A (2012b) Mycorrhiza-induced resistance in banana acts on nematode host location and penetration. *Soil Biol Biochem* 47:60–66
- Vos CM, Yang Y, De Coninck B, Cammue BPA (2014) Fungal (-like) biocontrol organisms in tomato disease control. *Biol Control* 74:65–81
- Vu TT (2005) Modes of action of non-pathogenic *Fusarium oxysporum* endophytes for bio-enhancement of banana toward *Radopholus similis*. PhD thesis, University of Bonn
- Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, von Wettstein D, Franken P, Kogel KH (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance disease resistance and higher yield. *Proc Natl Acad Sci U S A* 102:13386–13391
- Whipps JM (2004) Prospects and limitations for mycorrhiza in biocontrol of root pathogens. *Can J Bot* 82:1198–1227
- Wubben MJ, Jin J, Baum TJ (2008) Cyst nematode parasitism of *Arabidopsis thaliana* is inhibited by salicylic acid (SA) and elicits uncoupled SA-independent pathogenesis-related gene expression in roots. *Mol Plant-Microbe Interact* 21:424–432
- Ye W, Shen CH, Lin Y, Chen PJ, Xu X, Oelmüller R, Yeh KW, Lai Z (2014) Growth promotion-related miRNAs in *Oncidium* orchid roots colonized by the endophytic fungus *Piriformospora indica*. *PLoS One* 9:e84920
- Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. *Mol Plant-Microbe Interact* 25:139–150
- Zuccaro A, Lahrman U, Guldener U, Langen G, Piffi S, Biedenkopf D, Wong P, Samans B, Grimm C, Basiewicz M, Murat C, Martin F, Kogel KH (2011) Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. *PLoS Pathog* 7(10). doi:10.1371/journal.ppat.1002290

Chapter 4

Mycorrhizal Fungi Under Biotic and Abiotic Stress

Manoj Kumar, Ram Prasad, Vivek Kumar, Narendra Tuteja,
and Ajit Varma

Abstract Mycorrhizal fungi are associated with host plant roots which complement and augment plant growth, productivity, and immunity; nevertheless, current work by scientists shows that mycorrhiza also provoke so-called induced systemic tolerance (IST) to abiotic and biotic stresses. As we discuss here, the mycorrhiza also upsurge nutrient uptake and transport from soils, thus reducing the need for chemical fertilizers and avoiding the buildup of nitrates and phosphates in the agricultural soils. A decrease in fertilizer use would reduce the effects of contamination of water from fertilizer run off and leaching lead to savings for farmers. Abiotic stresses (such as soil salinity, drought, heat, cold, mineral deficiency) have become main threats to the universal agricultural production. These stress in alone and/or in combination control the plant growth, development, maturity, and productivity by causing physiological disorders, ion toxicity, and nutritional and hormonal disparities. Some precious soil microbes like mycorrhizal fungi inhabit the rhizosphere and develop a symbiotic and mutualistic relationship with the roots of most host plant species. Mycorrhiza can considerably enhance resistance of host plants to varied abiotic and biotic stresses. In this chapter, we highlight the importance of mycorrhizal fungi alleviation of various stresses and their beneficial effects on plant growth expansion and production. Though these stresses can negatively affect the mycorrhizal fungi, there are many reports which exhibit better growth, performance, and production of mycorrhizal plants under stress conditions. These positive consequences are explained by increased host plant nutrition, higher potassium, nitrogen and phosphate in plant tissues and a better osmotic modification by buildup of well-matched solutes such as proline, glycine betaine, or soluble sugars. Mycorrhizal inoculated plants also increase photosynthetic, physiological, biological, and water use efficiency under various stresses.

M. Kumar (✉) • R. Prasad • V. Kumar • N. Tuteja • A. Varma
Amity Institute of Microbial Technology, Amity University, Sector-125, Noida 201303,
Uttar Pradesh, India
e-mail: mkumar9@amity.edu

4.1 Introduction

Being sessile in nature, plants have a greater chance to interact with their immediate environment. In particular, conditions with abiotic stress factors like salinity, drought, cold, heat, nutrient imbalances, and metals can severely impact growth and development of plants and finally decrease their overall yield to about 70% (Auge 2001; Saxena et al. 2013). In order to avoid stresses and minimize their potential impacts, plants may bring several modifications in their morphology and/or structure/ultrastructure (Souza et al. 2012). Alternatively, plants may adopt several stress tolerance strategies through modulating their physiology and biochemistry to limit stress accrued damages or to facilitate the repair of damaged systems (Sharifi et al. 2007). Notably, adoption of above-mentioned strategies by plants can be modulated to achieve improved plant productivity/yield by externally applying chemicals and other sustainable efforts (Siqueira et al. 1990). Among the sustainable efforts, the association of soil microbiota with plant roots can also be exploited and implied to improve plant growth and productivity under normal and stressful environment (Talavera et al. 2001; Sailo and Bagyaraj 2005; Marulanda and Barea 2009). Arbuscular mycorrhizal fungi (AMF) are microscopic filamentous fungi that colonize the roots and their rhizosphere simultaneously and spread out over several centimeters in the form of ramified filaments (Toth et al. 1990; Xie et al. 2014). Mycorrhizal fungi are the most extensively studied fungal symbionts which are associated with approximately 90% of all land plants and has been reported to significantly contribute multiple benefits to its host plants (Muthukumar et al. 2014; Prasad et al. 2017). The AM fungi are of great ecological implication as they can form beneficial symbiosis with the most terrestrial plants (Xie et al. 2014) and also with a few wetland or swamp plants (Xie et al. 2014). Literature is full on the role of AM fungi in improving plant growth, metabolism, and eventually bring high crop/plant productivity under normal and stressful environment (Abdel Latef and Chaoxing 2011; Gavito and Azón-Aguilar 2012; Impa et al. 2012; Beltrano et al. 2013; Gholamhoseini et al. 2013).

Indeed, survival necessitates the ability to rapidly adapt to changes in the environment, especially those which represent long term or chronic changes. Whenever possible, one of the easiest ways to counteract such stresses is to relocate to a more suitable niche. However, such a strategy is obviously restricted in a short-term period and is not achievable with stationary organisms such as plants. Consequently, plants have developed a variety of strategies to cope against biotic stresses such as herbivory or parasitism and abiotic stresses such as salinity, drought, heat, or toxic metal contamination (Khan 1974; Parida et al. 2002; Sowinski et al. 2005; Kumar et al. 2010). Among abiotic stresses, soil salinization is probably one of the most important in the world (Sharifi et al. 2007; Aroca et al. 2013; Talaat and Shawky 2014). In addition, high temperature and low precipitation leading to salt accumulation at the soil surface affect the establishment, growth, and development of plants and even more as salinity increases. The delay in root growth can be caused by too low soil water potential and salt cell toxicity (Beltrano et al. 2013).

The cell toxicity causes cell death and also root necrosis in very sensitive plant genotypes. In tally to all these harmful effects on roots, shoot growth is also influenced and as a result the root/shoot ratio is troubled (Maggio et al. 2007). Overall, salinity leads to many deleterious effects on plants and that at different life stages. To counteract this problem, many strategies were proposed to overcome salt detrimental effects such as searching for new salt tolerant crops, genetically engineering plants, removing excessive salt accumulation in groundwater and desalinating water for irrigation. Although these strategies appear efficient, yet they are costly and out of reach for developing countries that are the most affected.

In this context, the ecosystem services rendered by soil biota in maintaining soil quality, plant health, and soil resilience are extremely pertinent (Gianinazzi and Gianinazzi Pearson 1988). In particular, soil microorganisms that form mutually beneficial relationships with plant roots have become a target of increasing interest in agricultural research and development because they offer a biological alternative to promote plant growth and reduce inputs in sustainable cropping systems (Gianinazzi et al. 2008). The ubiquity of AM fungi at the interface between soil and plant roots makes them a key functional group of soil biota which by their nutritional and non-nutritional activities profoundly influences ecosystem processes that contribute to the ecosystem services in agro-ecology. Our aims in this chapter are to highlight the key role that the mycorrhizal symbiosis can play as an ecosystem service provider to guarantee plant productivity and quality under abiotic and biotic stress conditions. The appropriate management of various stresses rendered by mycorrhiza will impact on natural resource preservation and utilization with an apparent net reward for human society.

4.2 Mycorrhiza and Abiotic Stresses

The abiotic stresses such as soil salinity and drought cause widespread losses to agricultural production. On the other hand, depletion of mineral, water stress, soil salinity and alkalinity, presence of heavy metals, or high temperature are serious problems in many parts of the globe, particularly in the arid and semi-arid regions (Evelin et al. 2009). It is forecasted that two-thirds of the cultivable agriculture land may vanish in Africa, a third in the Asia, and one-fifth in the South America by the year 2025. It is also predicted that the arable land area per occupant or resident on the planet will be lessened to 0.15 ha in the year 2050 (<http://www.un.org/esa/sustdev/documents/agenda21/french/action12.htm>). In countries like United States and Spain, one-third of the country part is undergoing desertification, which is an alarming situation.

The potential and probability of mycorrhizal fungi to improve the plant tolerance in abiotic stress conditions has been recognized since long (Smith and Read 2008), and their manipulation and application in perpetual and lasting agricultural systems will be of incredible and remarkable importance for soil quality and crop production under severe edapho-climatic conditions (Lal 2009). Among more current

examples of the employment of valuable and advantageous soil microbes to augment crop tolerance against several abiotic stress conditions. Studies on the beneficial effect of co-inoculated bacteria and mycorrhizal fungi from arid environments on plant growing under drought stress (Marulanda-Aguirre et al. 2008; Marulanda and Barea 2009) emphasize the interest of influencing autochthonous or natural mycorrhizal fungal isolates from the dry regions for re-vegetation of the degraded land zones to enhance soil quality, productivity, and to fight against desertification in the Mediterranean ecosystems. This is justified by quoting an example of an indigenous drought tolerant strain of *Glomus intraradices* associated with innate and local bacterium reduced by 42% the water required for production of *Retama sphaerocarpa* (Marulanda et al. 2006). In another report (Bouamri et al. 2006; Porrás-Soriano et al. 2009), the mycorrhizal fungi alleviate stress salinity in olive tree plantations in Spain or in arid North African region, where palm grove yields are considerably influenced by water drought and soil salinity conditions.

Alternative area where mycorrhizal fungal inoculation has become a potential and probable tool for increasing plant tolerance to the environmental stress conditions is in the re-vegetation of naturally or industrially deliberately metal contaminated soils. There are several examples in the literature to demonstrate and exemplify this role of mycorrhizal symbiosis, though the fundamental mechanisms are not hitherto fully understood (Khade and Adholeyan 2009). Occurrence of mycorrhizal fungi in Ni hyper accumulating plant species found naturally on the metal rich soils proposes potentials of using heavy metal hyper accumulating plants along with mycorrhizal fungi for phytoremediation approaches and tactics (Turnau and Mesjasz-Przybyłowicz 2003; Gamalero et al. 2009). Additionally, in another reports conducted by Lugon-Moulin et al. (2006) and Nziguheba and Smolders (2008), many phosphate fertilizers are a chief source of soil pollution by Cd in agricultural systems which again pleads for the lessening of crop dependence on phosphate fertilizers. Rivera-Becerril et al. (2002) and López-Millán et al. (2009) reported that mycorrhizal fungi, through their mycelium network, not only enhance inorganic phosphate uptake by plant roots but they also have a buffering consequence on the Cd uptake, decreasing the toxic effect of Cd on plant growth and production.

4.3 Mycorrhiza and Drought Stress

Drought, also commonly known as water deficiency or water stress, is the absence of satisfactory water table for normal plant development and growth (Subramanian and Charest 1998, 1999; Marulanda and Barea 2009). The unobtainability or inaccessibility of water to the root zone, stern transpiration rate or accelerated generation of reactive oxygen species (ROS), and consequent initiation of oxidative stress in plants can be key reasons of drought stress effects in plants (Auge 2001; Bárzana et al. 2012). Symbiosis of plant with mycorrhiza can progress the overall plant growth by enhancing root thickness and length, leaf area, plant biomass, and

nutrient uptake and transport under mild to severe drought condition (Davies et al. 2002). Enhancement of plant growth by mycorrhizal inoculation can be attributed to the formation of widespread hyphal networks and excretion of glomalin, which in turn augment water and micro- and macronutrients uptake, thus improving soil structure (Gholamhoseini et al. 2013).

Participation of mycorrhizal symbiosis in numerous biochemical and physiological processes including (1) direct uptake and transport of water and nutrients by mycorrhizal fungi, (2) augmented osmotic regulation, (3) enhanced gas exchange and water use effectiveness, and (4) better defense against oxidative damage has also been reported in literature (Mittler 2002; Marulanda et al. 2007; Ruíz-Sánchez et al. 2010). Mycorrhizal symbiosis resulted in superior and better leaf water potential, enhanced gas exchange, augmented stomatal conductance and transpiration and photosynthetic rates in mycorrhizal inoculated plants under drought conditions (Morte et al. 2000; Mena-Violante et al. 2006).

Mycorrhizal fungi can also alter water regulation in the plant through alteration in hormonal balance signaling or by motivating osmolytes in the mycorrhizal plants (higher strength or amount of photosynthetic by products and solvable sugars in the leaf symplasm) compared to non-mycorrhizal plants (Porcel and Ruiz-Lozano 2004). In this regard, role of abscisic acid (ABA) has also been proposed as one of the non-nutritional instrument by which mycorrhizal symbiosis stimulates stomatal conductance and other physiological characters in drought stressed exposed plants (Porcel et al. 2006; Ruiz-Lozano et al. 2006). Recently, in *Zea mays* plants colonized by mycorrhiza *Glomus intraradices*, an augmented expression of two aquaporin genes (GintAQPF1 and GintAQPF2) was described in both root cortical cells holding arbuscules and extra radical mycelia under the drought stress (Moussa and Abdel-Aziz 2008; Li et al. 2013). Porcel et al. (2007), Li et al. (2013) and Rapparini and Peñuelas (2014) reported that mycorrhizal hyphal growth was also found to be connected with water absorption area.

4.4 Mycorrhiza and Nutrients Stress

Insufficiency of mineral micro- and macronutrients has been reported to affect plant growth by persuading changes in the plant growth pattern, chemical composition, and antioxidant defense capacity ultimately rendering plant susceptibility to diverse stress aspects (Hajiboland 2012). Remarkably, mycorrhizal infection can progress the uptake of micronutrients and other macronutrients having low mobility including Fe, Cu, and Zn (Baslam et al. 2013). The mycorrhizal inoculated lettuce plants with higher accessibility of nitrogen and phosphorus in soil revealed decreased content of micro- and macronutrients in the tissues (Azcón et al. 2003). Mycorrhizal colonization also enhanced all the micro- and macronutrients when plants were fertilized with a low level of both phosphorus and nitrogen (Baslam et al. 2013; Ortas and Ustuner 2014; Xie et al. 2014). In another report, Yaseen et al. (2012) recorded highest macro- and micronutrients (Ca, K, Mg, P, Fe, and Si) uptake of

chickpea in mycorrhizal inoculated plants. Development of widespread hyphal network in the soil improves effects of exceptionally low pH through improved uptake of phosphorus (Muthukumar et al. 2014). About 80% of the total phosphorus acquired by mycorrhizal *Medicago truncatula* was delivered by the extra radical mycelium of the mycorrhizal fungi connected with those plants (Smith et al. 2000). Also, Rohyadi (2008) perceived an upsurge in phosphorus uptake and transport in maize colonized by mycorrhiza *G. margarita* under acidic conditions and advocated that augmented phosphorus levels in mycorrhizal maize tissues could be owing to the better assessment of soil by mycorrhizal fungal hyphae (Muthukumar et al. 2014).

4.5 Mycorrhiza and Biotic Stresses

To check the spread of pests (pathogenic bacteria, fungi, virus, and nematodes) causing great yield losses in common cultivated crops, usual agriculture practice has been using huge quantities of harmful pesticides. Along with this, scientists are working on the plant breeding programs in order to obtain disease resilient plants. Nevertheless, the pesticides are often only partly or moderately effective against potential soil-borne diseases. Furthermore, they are disadvantageous and unfavorable to human health and also to the environment and as a result an ever cumulative number of pesticides are being taken off the market. Additionally, disease resistance obtained by plant breeding plans is often due to single plant genes, which can be quickly weakened by evolving biodiversity in the pathogenic agents. Balancing tactics have therefore to be developed to guarantee durable tolerance of plants to the potential pathogens.

Several studies have confirmed the beneficial effect of mycorrhizal fungi in encouraging plant tolerance to biotic stress triggered by soil-borne potential pathogens interrelating with many plant species. This has been constantly demonstrated among a number of pathogenic fungi or Oomycetes such as *Fusarium*, *Rhizoctonia*, *Verticillium*, *Thievalopsis*, *Aphanomyces*, *Phytophthora*, and *Pythium*, as well as the nematodes from the genera *Heterodera*, *Meloidogyne*, *Pratylenchus*, and *Radopholus* (Harrier and Watson 2004; Whipps 2004; Hao et al. 2009). Most of the research work has been carried out under very precise and planned conditions at the early stages of plant growth, but a few studies conducted in field or in greenhouse under actual production conditions approve these results (Bødker et al. 2002; Newsham et al. 1995; Torres-Barragan et al. 1996; Utkhede 2006). It would not be possible to write here all the research papers published on this topic. In its place, we have selected to exemplify this by results attained for tomato crop which is one of the most extensively grown vegetables all over the world. Moreover, this crop is susceptible to many bacteria, insects, fungi, and nematodes causing noteworthy reduction in fruit yield (34%) under current production practices, as reported by (Engindeniz 2006). Though the tomato plant is not highly approachable to mycorrhizal fungi in terms of plant growth (Smith et al. 2009), but

it clearly reimbursements from mycorrhiza inoculation when challenged by plant root pathogens such as *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Rhizoctonia solani*, *Phytophthora parasitica*, or *Meloidogyne incognita*. In this case, root colonization by mycorrhizal fungi can principally lessen root infection and disease seriousness caused by the potential pathogens, which results in enhanced fresh plant weight (up to 198%) and fruit yield (14.3%) compared to the pathogen infected mycorrhizal non-inoculated plants.

4.6 Mycorrhizosphere: A Biocontrol Zone

The mycorrhizosphere is zone covered and explored by mycorrhiza and has been hypothesized to constitute an environment conducive to microbes hostile to soil-borne potential pathogen survival and proliferation. Certainly, co-inoculation of the non-mycorrhizal species *Dianthus caryophyllus* with the mycorrhizal species *Tagetes patula* in the presence of *G. intraradices* evidently lessened the disease caused by *F. o. dianthi* in the *D. caryophyllus* in a way clearly unconnected to plant nutrition which proposes a lessening in pathogen growth and development within this mycorrhizosphere (St-Arnaud et al. 1997). Furthermore, a decrease in the number of infection loci of tomato roots pre-colonized with the *G. mosseae* and inoculated with *P. nicotianae* zoospores concludes that pathogen may be disturbed prior to root infiltration and penetration in mycorrhizosphere (Vigo et al. 2000).

Mycorrhizosphere influenced by the rhizobacteria–mycorrhiza–root tripartite association also presents precise characteristics, in which each character influences the other actor's growth, development, and health. Remarkably through the discharge of glycoproteins such as glomalin, mycorrhiza fungi favor the establishment of aggregates which provide stable microsites which are favorable to root and microbe establishment (Rillig and Mummey 2006). The mycorrhizal extra radical network also establishes specific microsites which also favor the growth of some bacteria. Amongst the plant growth promoting rhizobacteria (PGPR) (Bowen and Rovira 1999), phosphate solubilizing and nitrogen fixing bacteria have been shown to synergistically interrelate and cooperate with mycorrhizal fungi, increasing phosphate and nitrogen availability to plant and promoting its growth and development and probably favoring its capability to counter pathogen impact on plant growth and production (Johansson et al. 2004; Barea et al. 2005; Artursson et al. 2006; Lioussanne et al. 2009).

4.7 Mycorrhiza and Phytophagous Insects

The mycorrhizal standing of the plant can also manipulate insect and herbivore performance, but the magnitude and direction of the effect depend upon the feeding mode and lifestyle of the insect pathogen (Hartley and Gange 2009;

Koricheva et al. 2009). Many different researches cover an abundant range of mycorrhizal–plant–insect interactions under controlled or natural/field conditions. Upon a wide-ranging review of published data, Hartley and Gange (2009) established that, generally, mycorrhizal fungi have strong negative effects on rhizophagous insects, but the effects on insects feeding on shoot are weaker and more inconstant. Regardless of this inconsistency, few general patterns appear: generalist insects are frequently adversely influenced by mycorrhizal fungus, whereas specialist insects may often take advantage. Moreover, aphids usually perform better on mycorrhizal plants while the leaf chewing insects are generally negatively affected by the association.

Above-mentioned patterns may arise from differential impact of nourishing and defense aspects in insect pathogen. While the common insects are sensitive to the plant defense mechanisms and the specialist herbivores are likely to be able to evade the defenses of their host plant and remain unnoticed. As a result, the common or generalists insects may be affected by the improved defense capacity of mycorrhizal plants, while the specialists will evade the defenses and may get benefit from improved nutritious status of host plant. The negative effect on leaf chewer insects is likely related to their vulnerability to jasmonate or jasmomic acid dependent defenses (Peña-Cortés et al. 2004) potentiated in mycorrhizal inoculated plants. Additionally, mycorrhizal fungi can also have an impact on herbivores by influencing the performance and function of their predators and parasitoids: for example, in tomato plant, the volatile blends released by mycorrhizal plants can be more attractive to aphid parasitoids compared to those from non-mycorrhizal plants (Guerrieri et al. 2004).

4.8 Conclusions

We can conclude that mycorrhizal fungi are significant for improving plant tolerance to abiotic and biotic stresses, but also respond to various types of stresses individually of their host plant. The stresses affect the richness and community composition of mycorrhizal fungi. A change in diversity of mycorrhizal fungi will feed back into host plant community and will lead to corresponding changes in variety and leading plant species, and these responses will become sturdier with the climate changes, agriculture practices, and plant invasions. Mycorrhizal fungi are proficient in adapting to the abiotic and biotic environment which may or may not increase their mutualistic performance. Impact of ecological and evolutionary responses of mycorrhizal fungi to abiotic and biotic stresses is likely to become even more imperative for both natural and cultivable agricultural systems in the face of biotic stresses and climate changes, such as incursion by non-native species. Substantial progress has been made in understanding the role of mycorrhizal symbiosis in bestowing drought confrontation to plants, but dissimilar aspects still necessitate attention for unknottting new and unique metabolites and hidden metabolic pathways. The collected biochemical, physiological, and molecular data

based on classical means and methods will benefit from the various “omic” techniques and procedures and their combinations. An in depth examination using the progressive methodologies could help to clarify and explain the mechanisms of drought avoidance and/or tolerance induced by mycorrhizal symbiosis and to distinguish the abiotic and biotic stresses induced processes of the protective mechanisms regulated by mycorrhizal symbiosis.

Increasing our information on modifications of the plant physiology in mycorrhizal fungi, as well as in the biology of the potential insect attackers, is indispensable in order to define markers of stimulated resistance and to generate analytical models for the outcome of particular mycorrhiza-insect interactions. Additional challenge ahead is to decode the connections in plant responses to abiotic and biotic stresses. The experimental indications point to common controlling nodes in signaling pathways governing responses to abiotic and biotic stresses, and those nodes could be the target of biotechnological approaches or tactics for optimization of plant protection by mycorrhizas. Lastly, it is significant to consider mycorrhizal fungi in a multitrophic context, as the influence of the symbiosis on plant interactions may be modified by other organisms in the ecosystem.

References

- Abdel Latef AA, Chaoping H (2011) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. *Sci Hortic* 127:228–233
- Aroca R, Ruiz-Lozano JM, Zamarreño ÁM, Paz JA, García-Mina JM, Pozo MJ, López-Ráez JA (2013) Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. *J Plant Physiol* 170:47–55
- Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environ Microbiol* 8:1–10
- Auge RM (2001) Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11:3–42
- Azcón R, Ambrosano E, Charest C (2003) Nutrient acquisition in mycorrhizal lettuce plants under different phosphorus and nitrogen concentration. *Plant Sci* 165:1137–1145
- Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. *J Exp Bot* 56:1761–1778
- Bárzana G, Aroca R, Paz JA (2012) Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Ann Bot* 109:1009–1017
- Baslam M, Garmendia I, Goicoechea N (2013) Enhanced accumulation of vitamins, nutraceuticals and minerals in lettuces associated with arbuscular mycorrhizal fungi (AMF): a question of interest for both vegetables and humans. *Review. Agriculture* 3:188–209
- Beltrano J, Ruscitti M, Arango MC, Ronco M (2013) Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. *J Soil Sci Plant Nutr* 13:123–141
- Bødker L, Kjølter R, Kristensen K, Rosendahl S (2002) Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field grown pea. *Mycorrhiza* 12:7–12

- Bouamri R, Dalpé Y, Serrhini MN, Bennani A (2006) Arbuscular mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco. *Afr J Biotechnol* 5:510–516
- Bowen GD, Rovira AD (1999) The rhizosphere and its management to improve plant growth. *Adv Agronom* 66:1–102
- Davies FT Jr, Olalde-Portugal V, Aguilera-Gomez L (2002) Alleviation of drought stress of Chile ancho pepper (*Capsicum annum* L. cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico. *Sci Hortic (Amst)* 92:347–359
- Engindeniz S (2006) Economic analysis of pesticide use on processing tomato growing: a case study for Turkey. *Crop Prot* 25:534–541
- Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann Bot* 104:1263–1280
- Gamalero E, Lingua G, Berta G, Glick BR (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. *Can J Microbiol* 55:501–514
- Gavito M, Azón-Aguilar C (2012) Temperature stress in arbuscular mycorrhizal fungi: a test for adaptation to soil temperature in three isolates of *Funneliformis mosseae* from different climates. *Agri Food Sci* 21:2–11
- Gholamhoseini M, Ghalavand A, Dolatabadian A (2013) Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. *Agric Water Manag* 117:106–114
- Gianinazzi S, Gianinazzi Pearson V (1988) Mycorrhizae: a plant's health insurance. *Chim Oggi* 10:56–68
- Gianinazzi S, Huchette O, Gianinazzi-Pearson V (2008) New outlooks in mycorrhiza applications. In: Baar J, Estaun V, Ortas I, Orfanoudakis M, Alifragis D (eds) Proceedings of the COST870 meeting “Mycorrhiza application in sustainable agriculture and natural systems”, 17–19 Sept 2008, Thessaloniki, Greece, pp 20–22
- Guerrieri E, Lingua G, Digilio MC, Massa N, Berta G (2004) Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? *Ecol Entomol* 29:753–756
- Hajiboland R (2012) Effect of micronutrient deficiencies on plants stress responses. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer Science+Business Media, LLC, New York, pp 283–329
- Hao Z, Fayolle L, van Tuinen D, Gianinazzi-Pearson V, Gianinazzi S (2009) Mycorrhiza reduce development of nematode vector og Grapevine fanleaf virus in soils and root systems. In: Boudon-Padfiu E (ed) Extended abstract 16th meeting of ICVG, Dijon, France, pp 100–1001
- Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. *Pest Manag Sci* 60:149–157
- Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Ann Rev Entomol* 54:323–342
- Impa SM, Nadaradjan S, Jagadish SVK (2012) Drought stress induced reactive oxygen species and anti-oxidants in plants. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer Science+Business Media, LLC, New York, pp 131–134
- Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol Ecol* 48:1–13
- Khade SW, Adholeyan A (2009) Arbuscular mycorrhizal association in plants growing on metal-contaminated and non-contaminated soils. *Water Air Soil Pollut* 202:45–56
- Khan AG (1974) The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and of endogone spores in adjacent soils. *J Gen Microbiol* 81:7–14
- Koricheva J, Gange AC, Jones T (2009) Effects of mycorrhizal fungi on insect herbivores: a metaanalysis. *Ecology* 90:2088–2097

- Kumar A, Sharma S, Mishra S (2010) Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation, and mycorrhizal dependency of *Jatropha curcas* L. *J Plant Growth Regul* 29:297–306
- Lal R (2009) Soil degradation as a reason for inadequate human nutrition. *Food Secur* 1:45–57
- Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B (2013) First cloning and characterization of two functional aquaporin genes from an Arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* 197:617–630
- Lioussanne L, Beaugregard M-S, Hamel C, Jolicoeur M, St-Arnaud M (2009) Interactions between arbuscular mycorrhiza and soil microorganisms. In: Khasa D, Piché Y, Coughlan A (eds) *Advances in mycorrhizal biotechnology: a Canadian perspective*. NRC Press, Ottawa
- López-Millán AF, Sagardoy R, Solanas M, Abadía A, Abadía J (2009) Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. *Environ Exp Bot* 65:376–385
- Lugon-Moulin N, Ryan L, Donini P, Rossi L (2006) Cadmium content of phosphate fertilizers used for tobacco production. *Agron Sust Dev* 26:151–155
- Maggio A, Raimondi G, Martino A, De Pascale S (2007) Salt stress response in tomato beyond the salinity tolerance threshold. *Environ Exp Bot* 59:276–282
- Marulanda A, Barea JM (2009) Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. *J Plant Growth Regul* 28:115–124
- Marulanda A, Barea JM, Azcon R (2006) An indigenous drought tolerant strain of *Glomus intraradices* associated with a native bacterium improves water transport and root development in *Retama sphaerocarpa*. *Microb Ecol* 52:670–678
- Marulanda A, Porcel R, Barea JM, Azcón R (2007) Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought sensitive *Glomus* species. *Microb Ecol* 54:543–552
- Marulanda-Aguirre A, Azcon R, Ruiz-Lozano JM, Aroca R (2008) Differential effects of a *Bacillus megaterium* strain on *Lactuca sativa* plant growth depending on the origin of the arbuscular mycorrhizal fungus coinoculated: physiologic and biochemical traits. *J Plant Growth Regul* 27:10–18
- Mena-Violante HG, Ocampo-Jiménez O, Dendooven L (2006) Arbuscular mycorrhizal fungi enhance fruit growth and quality of chile ancho (*Capsicum annum* L. cv San Luis) plants exposed to drought. *Mycorrhiza* 16:261–267
- Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7:405–410
- Morte A, Lovisolo C, Schubert A (2000) Effect of drought stress on growth and water relations of the mycorrhizal association *Helianthemum almeriense*–*Terfezia clavervyi*. *Mycorrhiza* 10:115–119
- Moussa HR, Abdel-Aziz SM (2008) Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Aust J Crop Sci* 1:31–36
- Muthukumar T, Priyadharsini P, Uma E, Jaison S, Pandey RR (2014) Role of arbuscular mycorrhizal fungi in alleviation of acidity stress on plant growth. In: Miransari M (ed) *Use of microbes for the alleviation of soil stresses*, vol 1. Springer Science+Business Media, New York, pp 43–71
- Newsham KK, Fitter AH, Watkinson AR (1995) Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J Ecol* 83:991–1000
- Nziguheba G, Smolders E (2008) Inputs of trace elements in agricultural soils via phosphate fertilizers in European countries. *Sci Total Environ* 390:53–57
- Ortas I, Ustuner O (2014) Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake. *Sci Hortic* 166:84–90
- Parida A, Das AB, Das P (2002) NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a tree mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J Plant Biol* 45:28–36

- Peña-Cortés H, Barrios P, Dorta F, Polanco V, Sánchez C, Sánchez E, Ramírez I (2004) Involvement of jasmonic acid and derivatives in plant response to pathogen and insects and in fruit ripening. *J Plant Growth Regul* 23:246–260
- Porcel R, Ruiz-Lozano JM (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *J Exp Bot* 55:1743–1750
- Porcel R, Aroca R, Cano C et al (2006) Identification of a gene from the arbuscular mycorrhizal fungus *Glomus intraradices* encoding for a 14-3-3 protein that is up-regulated by drought stress during the AM symbiosis. *Microb Ecol* 52:575–582
- Porcel R, Aroca R, Cano C, Bago A, Ruiz-Lozano JM (2007) A gene from the arbuscular mycorrhizal fungus *Glomus intraradices* encoding a binding protein is up regulated by drought stress in some mycorrhizal plants. *Environ Exp Bot* 60:251–256
- Porrás-Soriano A, Soriano-Martin ML, Porrás-Piedra A, Azcon R (2009) Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J Plant Physiol* 166:1350–1359
- Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) *Mycorrhiza*. Springer, Cham, pp 1–7
- Rapparini F, Peñuelas J (2014) Mycorrhizal fungi to alleviate drought stress on plant growth. In: Miransari M (ed) *Use of microbes for the alleviation of soil stresses*, vol 1. Springer Science +Business Media, New York, pp 21–42
- Rillig MC, Mummy DL (2006) Mycorrhizas and soil structure. *New Phytol* 171:41–53
- Rivera-Becerril F, Calantzis C, Turnau K, Caussanel JP, Belimov AA, Gianinazzi S, Strasser RJ, Gianinazzi-Pearson V (2002) Cadmium accumulation and buffering of cadmium induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes. *J Exp Bot* 53:1177–1185
- Rohyadi A (2008) Growth responses of external hyphae of arbuscular mycorrhizal fungi to acidic soil conditions and their effects on cowpea growth. *Microbiol* 2:22–26
- Ruiz-Lozano JM, Porcel R, Aroca R (2006) Does the enhanced tolerance of arbuscular mycorrhizal plants to water deficit involve modulation of drought-induced plant genes? *New Phytol* 171:693–698
- Ruiz-Sánchez M, Aroca R, Muñoz Y, Armada E, Polón R, Ruiz-Lozano JM (2010) The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. *J Plant Physiol* 167:862–869
- Sailo GL, Bagyaraj DJ (2005) Influence of different AM fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*. *Mycol Res* 109:795–798
- Saxena S, Kaur H, Verma P, Petla BP, Andugula VR, Majee M (2013) Osmoprotectants: potential for crop improvement under adverse conditions. In: Tuteja N, Gill SS (eds) *Plant acclimation to environmental stress*. Springer Science+Business Media, New York, pp 197–232
- Sharifi M, Ghorbanli M, Ebrahimzadeh H (2007) improved growth of salinity stressed soybean after inoculation with pre-treated mycorrhizal fungi. *J plant physiol* 164:1144–1151
- Siqueira JO, Rocha WFJR, Oliveira E, Colozzi-Filho A (1990) The relationship between vesicular-arbuscular mycorrhiza and lime: Associated effects on the growth and nutrition of brachiaria grass (*Brachiaria decumbens*). *Biol Fertil Soil* 10:65–71
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic Press, London
- Smith FW, Rae AL, Hawkesford MJ (2000) Molecular mechanisms of phosphate and sulphate transport in plants. *Biochem Biophys Acta* 1465:236–245
- Smith FA, Grace EJ, Smith SE (2009) More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytol* 182:347–358
- Souza LA, Andrade SAL, Souza SCR, Schiavinato MA (2012) Evaluation of mycorrhizal influence on the development and phytoremediation potential of *Canavalia gladiata* in Pb contaminated soils. *Int J Phytoremed* 15:465–476

- Sowinski P, Rudzinska-Langwald A, Adamczyk J, Kubica I, Fronk J (2005) Recovery of maize seedling growth, development and photosynthetic efficiency after initial growth at low temperature. *J Plant Physiol* 162:67–80
- St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA (1997) Inhibition of *Fusarium oxysporum* f.sp. *dianthi* in the non-VAM species *Dianthus caryophyllus* by co-culture with *Tagetes patula* companion plants colonized by *Glomus intraradices*. *Can J Bot* 75:998–1005
- Subramanian KS, Charest C (1998) Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. *Physiologia Plantarum* 102:285–296
- Subramanian KS, Charest C (1999) Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions. *Mycorrhiza* 9:69–75
- Talaat NB, Shawky BT (2014) Protective effects of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) plants exposed to salinity. *Environ Exp Bot* 98:20–31
- Talavera M, Ito K, Mizukubo T (2001) Reduction of nematode damage by root colonization with arbuscular mycorrhiza (*Glomus* spp.) in tomato *Meloidogyne incognita* (Tylenchida: Meloidogyndae) and carrot-*Pratylenchus penetrans* (Tylenchida: Pratylenchidae) pathosystems. *Appl Entomol Zool* 36:387–392
- Torres-Barragan A, Zavaleta-Mejia E, Gonzalez-Chavez C, Ferrera-Cerrato R (1996) The use of arbuscular mycorrhizae to control onion white rot (*Sclerotium cepivorum* Berk.) under field conditions. *Mycorrhiza* 6:253–257
- Toth R, Toth D, Starke D, Smith DR (1990) Vesicular-arbuscular mycorrhizal colonization in *Zea mays* affected by breeding for resistance to fungal pathogens. *Can J Bot* 68:1039–1044
- Turnau K, Mesjasz-Przybylowicz J (2003) Arbuscular mycorrhiza of *Berkheya codii* and other Ni-hyper accumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza* 13:185–190
- Utkhede R (2006) Increased growth and yield of hydroponically grown greenhouse tomato plants inoculated with arbuscular mycorrhizal fungi and *Fusarium oxysporum* f. sp. *radicislycopersici*. *Biocontrol* 51:393–400
- Vigo C, Norman JR, Hooker JE (2000) Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant Pathol* 49:509–514
- Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Can J Bot* 82:1198–1227
- Xie X, Weng B, Cai B, Dong Y, Yan C (2014) Effects of arbuscular mycorrhizal inoculation and phosphorus supply on the growth and nutrient uptake of *Kandelia obovate* (Sheue, Liu & Yong) seedlings in autoclaved soil. *Appl Soil Ecol* 75:162–171
- Yaseen T, Burni T, Hussain F (2012) Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth and productivity of chickpea (*Cicer arietinum*) varieties. *Int J Agron Plant Prod* 3:334–345

Chapter 5

Role of Arbuscular Mycorrhizal Fungi (AMF) in Salinity Tolerance and Growth Response in Plants Under Salt Stress Conditions

Mahesh Borde, Mayura Dudhane, and Mohan Kulkarni

Abstract Salt stress is a major agricultural problem all over the world, which has an effect on the functioning of growth and physiology of crop plants. Salinity in soil reduces plant growth by decreasing net photosynthesis rate and stomatal conductance; it also inhibits antioxidant enzyme index. Arbuscular Mycorrhizal Fungi (AMF) are one of the root symbionts which play a vital role in enhancing the crop plant growth and help the host plant in developing tolerance against abiotic stress factors like salt, drought, etc. This chapter aims to evaluate the beneficial role of AMF on plant growth and physiological performance under salinity stress conditions. AMF help to enhance the plant growth, by increasing plant biomass, photosynthetic activity water potential, and selective uptake of nutrients under salinity stress condition. AMF reduce the adverse effect of salt stress by increasing antioxidant defense mechanism in response to salinity stress conditions and promote salinity tolerance in crop plants. AMF mitigate the salt-induced deleterious effects by virtue of maintaining the osmotic balance by regulating the Na^+ and K^+ ratio. AMF also help in maintaining osmotic adjustment in host plants by inducing the synthesis of various osmolytics like proline, glycine betaine, etc. Thus, tolerant AMF species can be used as bioinoculant to improve agricultural productivity under salinity stress conditions.

5.1 Introduction: Effect of Soil Salinity on Growth of Plant

Soil salinity is a major problem in numerous parts of the world in arid and semiarid regions (Giri et al. 2003; Al-Karaki 2006). Also, 7% of the earth's land surface has become saline prone (Ruiz-Lozano et al. 2001). Excessive application of chemical

M. Borde • M. Dudhane
Department of Botany, Savitribai Phule Pune University, Pune 411007, Maharashtra, India

M. Kulkarni (✉)
Division of Biochemistry, Department of Chemistry, Savitribai Phule Pune University, Pune 411007, India
e-mail: drmvkulkarni@gmail.com

fertilizers, use of ground water for irrigation, flood irrigation practices, and no rotation of crops are the major reasons for increasing agricultural soil salinization. Agricultural productivity is affected, if the fertile soil is transformed into saline soil, as increased soil fertility decreases up to 20% of crop productivity (Porcel et al. 2012; Munns and Gilliam 2015). Increased soil salinization of arable land results in the loss of 30% of agricultural land within next 25 years and up to 50% within next 40 year (Porcel et al. 2012; Abdel-Fattah et al. 2014). High salt accumulation in soil decreases soil porosity, soil aeration, and water conductance which results in water deficit condition to the plant and causes physiological drought (Mahajan and Tuteja 2005). Salinity injuries could decrease photosynthetic rate, reduce antioxidant enzyme activities, reduce stomatal conductance, induced ion deficiencies, affect membrane stability index, and reduce relative water content of the plants (Talaat and Shawky 2012). In addition to these factors, some other factors like physiological drought, ion toxicity, ion imbalance, and soil compaction may cause growth reduction. Salinity adversely affects the normal growth of plant by causing injury of foliage, nutrient deficiencies, lowering soil properties, nitrate content, inhibiting carbonic anhydrase, and nitrate reductase activities. Salt stress destroys the PSII reaction center and disrupts electron transport in the photosynthetic apparatus which reduces the net photosynthetic rate (Sheng et al. 2008; Dudhane et al. 2011; Talaat and Shawky 2012).

Also, soil salinity affects plant growth through toxic effects of Na^+ and Cl^- ion, which leads to denature enzyme structure, damage to cell organelles, decreased photosynthesis, respiration, and disturbing osmotic imbalance leading to physiological drought, as well as nutrient imbalance in the plant. So, collectively many such effects due to salt stress ultimately lead to reduced plant growth as well as loss in agricultural productivity (Adiku et al. 2001; Ramoliya et al. 2004; Evelin et al. 2009).

In saline soil, plant cells take up large amount of dissolved salts which results in plasmolysis of plant cells, and these cells start to collapse, affecting the morphological parameters of the plants like leaf expansion and reduction in fresh and dry matter and content of leaf and root tissue (Hernandez et al. 1995). There are some reports which show that the low level of salinity does not affect the plant growth of legumes, but plant weight, fresh, and dry matter content decreases significantly (Parida and Das 2005). The shoot and root length and dry biomass content of *Trifolium alexandrinum* were also decreased after salinity stress condition (Shokri and Maadi 2009). Many studies have been carried out on variety of plants to understand the detrimental effect of osmotic stress created by soil salinity. Salinity is also responsible for oxidative damage to the plant through the generation of reactive oxygen species (ROS) (Ahmad et al. 2010). In response to salt stress, plants accumulate different types of salt stress proteins and osmolytes like proline, glycine betaine, and malonaldehyde to protect the plant from osmotic shock. These biochemical constituents are accumulating in higher concentration after salt stress exposure.

5.2 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) is special type of symbiotic fungus which forms symbiotic, nonpathogenic association with the terrestrial plant root. The AMF are widely distributed in saline land of terrestrial ecosystem (Yamato et al. 2008). This type of plant fungus symbiotic partnership is found in a wide range of plants including angiosperm, gymnosperm, and pteridophytes; mosses; etc. (Bago et al. 2000). This symbiotic association improves water and nutrient uptake of host plant and protects the plant from various biotic and abiotic stresses (Gupta et al. 2000; Zuccarini and Okurowska 2008). AMF are soil fungi belonging to a recently new approved phylum, Glomeromycota, with a presumed origin at least 460 million years ago. AMF are obligate symbionts that require plant hosts to complete their life cycle. In soil, their spores germinate during favorable conditions and recognize compatible host roots to activate their symbiotic relationship (Schubler et al. 2001; Prasad et al. 2017).

AMF are effective to plants performance against different salinity stress conditions. Under 100 mM NaCl salt stress, AMF-inoculated *Allium sativum* plant showed increased morphological parameters like leaf area, plant fresh weight, and dry weight (Borde et al. 2010). The increase in biomass of tomato plant inoculated with *Glomus mosseae* was observed under moderately saline condition (Ghazi and Al-Karaki 2001). Several studies have been carried out to investigate the role of AMF-inoculated plant in adaptation against salt stress. The positive cumulative effects like nutrient uptake, photosynthetic ability, and biochemical and physiological performance on plant growth due to mycorrhizal inoculation mitigate the salt tolerance (Borde et al. 2011). Under salt stress condition, several researchers showed that the AMF-inoculated plant increased their fitness than non-AMF-inoculated plant (Sannazzaro et al. 2007; Zuccarini and Okurowska 2008; Borde et al. 2011). Mycorrhization improved higher biomass and fruit yield in tomato plant under salt stress (Al-Karaki 2000). Many studies on various plants have demonstrated that mycorrhizal fungi help the plant under salinity stress conditions by enhancing photosynthetic ability, uptake of various nutrients, increasing antioxidant defense, and increasing osmolyte accumulation which results in improved plant growth and tolerance under salinity stress condition (Table 5.1). Increase in the growth parameters of the host plants have been observed in all these studies (Sharifi et al. 2007). In various greenhouse experiments, different AMF species like *Glomus fasciculatum*, *Glomus mosseae*, *Glomus etunicatum*, and *Glomus intraradices* were tested for improvement of growth parameters like shoot length, root length, fresh and dry weight, number of nodules, leaf number, pod number, etc. (Kaya et al. 2009).

Table 5.1 Effects of AMF on plant performance under salinity stress conditions

Sr No.	Plant name	Effect of AMF	Reference
1.	<i>Solanum Lycopersicon</i>	Enhanced chlorophyll content	Hajiboland et al. (2010)
		Increased antioxidant activity	He et al. (2007), Ghorbanli et al. (2004), Qun et al. (2007)
2.	<i>Lactuca sativa</i>	Increased in growth, minerals content	Kohler et al. (2009)
3.	<i>Piper nigrum</i>	enhanced chlorophyll content	Kohler et al. (2009)
4.	<i>Citrus sinensis</i>	Uptake of potassium and calcium	Wu et al. (2010)
5.	<i>Oscimum basilium</i>	Increased in proline accumulation	Shekoofeh et al. (2012)
6.	<i>Phragmites australis</i>	Higher osmolytes accumulation	Al-Garni (2006)
7.	<i>Cajanus cajan</i>	Higher osmolytes accumulation	Garg and Manchanda (2009)
8.	<i>Zea Mays</i>	Higher osmolytes accumulation	Sheng et al. (2011)
		Increased water potential and photosynthetic activity	
9.	<i>Glycine max</i>	Increased CAT activity	Ghorbanli et al. (2004)
10.	<i>Trifolium alexandrinum</i>	Increased plant biomass	Shokri and Maadi (2009)
11.	<i>Acacia nilotica</i>	Increased Potassium	Giri and Mukerji (2004)
12.	<i>Sesbania aegyptiaca</i>	Increased in chlorophyll content and potassium	Giri et al. (2007)
13.	<i>S. grandiflora</i>	Decreased in potassium	Wu et al. (2010)
14.	<i>Cucurbita pepo</i>	Enhanced relative water content	Colla et al. (2008)
15.	<i>Vigna radiate</i>	Higher plant growth	Rabie (2005)

5.3 Salinity Tolerance by AMF Through Nutrient Uptake and Ionic Balance

AMF may increase the tolerance of plants to salinity stress by providing nutrient uptake, and the selective absorption of ion leads to ion balance during stress condition (Asghari et al. 2005; Wu et al. 2010; Hammer et al. 2011). Also, AMF protect the plants by activating some enzymes (Giri and Mukerji 2004; Rabie and Almadini 2005) and alleviate water stress (Sheng et al. 2008). AMF have been shown to promote plant growth; enhance nutrient uptake such as Nitrogen, Phosphorus, Magnesium, and micronutrients from the soil; improve soil structure and also able to enhance plant tolerance under different abiotic and biotic stresses such as drought and salinity; and protect host plants against pathogens (Wu et al. 2014; Hameed et al. 2014; Hashem et al. 2014b; Evelin et al. 2012; Sikes et al. 2009). Crop plants inoculated with AMF have been shown to enhance the plant growth and agricultural yield and maintain the osmotic and ionic adjustment to a normal level so that crop plants will grow well under salinity stress conditions (Hameed et al.

2014). This AMF association improves absorption of water and nutrient uptake, solubilizes the complex into existing forms, and enhances the nutrient profile and growth profile of host plant. AMF also help in nutrient cycling in soil, root architecture, and enables to provide essential nutrients to host plant under the salinity stress. AMF play a key role in the regulation of ion and membrane transport proteins that control the ion homeostasis of the host plants (Ramos et al. 2011).

Increase in AMF colonization leads to Phosphorus (P) uptake in *Pennisetum glaucum* plants which indicates that alkaline phosphatases are probably involved in P acquisition, and there are possibilities that more than one acid phosphatase might be responsible for the transporting P, thus leading to increased P uptake under salt stress condition. A good amount of K/Na ratio is also considered to be beneficial for maintaining ion balance in the cytoplasm. The ability of the plant to tolerate stress mainly depends upon the amount of P accumulated.

Excess of sodium chloride (NaCl) causes damage to nearly about 20% of 230 million irrigated agricultural land in and around the world (Munns and Tester 2008). Higher concentration of Na^+ (>40 mM) have damaging impact on plant growth for the most part due to hyperosmotic stress (water deficit under strongly negative water potential), over absorption and imbalance of ion (Munns and Tester 2008). During salinity stress, ions like potassium (K^+) and sodium (Na^+) play an important role in contribution towards the strength of an ion and osmotic pressure (Serrano and Rodriguez Navarro 2001). Increased salinity in the irrigated agricultural land causes crop plant to decrease the concentration of K^+ , Ca^{2+} , NO_3^- and also increase the concentration of inorganic phosphate (Pi). Whereas, the concentration of Na^+ and Cl^- ions increases to such an extent that it leads to ionic injuries and osmotic and nutritional imbalance (Bothe 2012).

Exposure of crop plants to salt stress results impedes uptake of essential mineral elements. Increased Na^+ concentration within the root zone directly influences the uptake of several essential elements like K^+ and Na^+ that share antagonistic relationship with K^+ (Kohler et al. 2009). Under salinity stress, AMF inoculation decreases the uptake of Na^+ and increases uptake of K^+ ion concentration, as compared to non-AM plants, suggesting AMF-induced preferential loading of K^+ than Na^+ into the root (Hu and Schmidhalter 2005). Excess amount of Na^+ and Cl^- ions in saline soil disturbs ionic balance in soil solution and hampers its original potential, therefore uptake, transport, and utilization of essential nutrients are affected by salt stress (Roberts et al. 1984). Hammer et al. (2011) found that AMF can selectively take up elements such as K^+ and Ca^{2+} , which act as osmotic equivalents by avoiding uptake of toxic Na^+ ion, which lower the Na^+ ion concentration in plant cell under salinity stress condition. This could make AMF important for salinity stress alleviation for their host plant.

Restricted uptake of potassium and phosphorous is observed by Kohler et al. (2009) and Wu et al. (2010). AMF inoculation to Citrus plants under salinity stress considerably mitigated the deleterious impact on the uptake of essential elements like phosphorous, potassium, and calcium (Wu et al. 2010). AMF inoculation under salinity stress condition in Lettuce plants contributed significantly to growth maintenance by mediating enhanced uptake of essential mineral elements as compared

to the non-inoculated Lettuce plant (Kohler et al. 2009). AMF inoculation maintaining higher K/Na ratio is one of the strategies of AMF to mitigate stress-induced deleterious changes in plants (Wu et al. 2010; Tomar and Agarwal 2013).

Optimal concentration of K^+ in plant cell is essential for several important metabolic processes (Tomar and Agarwal 2013). Under salinity stress condition, AMF inoculation showed selective absorption of P, K, and Ca ion over deleterious Na^+ and maintaining lower Na/K ratio (Ahmad et al. 2014).

5.4 Salinity Tolerance by AMF Through Plant Physiological Response

Shi et al. (2002) and Shi and Guo (2006) found that salt stress could decrease photosynthetic ability and induce physiological drought in plants, which leads to a decrease in crop production. AMF are known to survive stressed soil and participate in the plant growth and development and improves the plant tolerance against biotic and abiotic stresses (Abd-Alla et al. 2000), by regulating the physiological and biochemical process of plants (Evelin et al. 2009). Recently, many researchers have reported that AMF could enhance ability of plants to cope with salinity stress (Yano-Melo et al. 2003; Rabie 2005; Jahromi et al. 2008) by improving plant nutrient uptake (Cantrell and Linderman 2001; Asghari et al. 2005) and ion balance (Giri et al. 2007), protecting enzyme activity (Rabie and Almadini 2005; Giri and Mukerji 2004), and facilitating water uptake (Berta et al. 1990; Ruiz-Lozano and Azcón 1995).

Rabie (2005) suggested that AMF protect the host plants against the detrimental effects of salinity stress. AMF showed higher growth performance in *Cajanus cajan* plants than non-AMF plants at all levels of irrigation. Also, there are reports of modifications of plant physiological performance of plants i.e., osmotic modifications (Rao and Tak 2002) and photosynthesis (Sheng et al. 2008; Borde et al. 2011). Biological remediation, such as the application of AMF to saline soils as bioinoculants, could alleviate salt stress in plants (Evelin et al. 2009; Porcel et al. 2012). This may be the result of a more efficient mineral uptake (Evelin et al. 2012), ion balance (Giri et al. 2007), protection of enzymatic activities (Patel and Saraf 2013), and/or facilitation of water uptake (Aroca et al. 2007).

Amount of chlorophyll pigments declines when affected by salinity stress. This reduced chlorophyll content caused by salinity stress was confirmed by Doganlar et al. (2010), Rasool et al. (2013), Datta and Kulkarni (2014), and Alqarawi et al. (2014) in *Solanum lycopersicum*, *Cicer arietinum*, *Glycine max*₂ and *Ephedra alata*, respectively. In *E. alata*, Alqarawi et al. (2014) demonstrated that chlorophyll content reduced considerably with increasing salinity levels. Reduced chlorophyll contents under stress is attributed to increased activity of chlorophyllase causing degradation of pigments and hence resulting in reduced photosynthesis and affect growth. Compared with stressed plants, AMF-inoculated plants maintained higher

contents of chlorophyll pigments. The fact that AMF colonization significantly increased chlorophyll content in many plants is supported by the findings of Hajiboland et al. (2010) for *Solanum lycopersicum* L. and Aroca et al. (2013) for lettuce. In pepper, inoculation of AMF increased chlorophyll content under normal as well as salt-stressed conditions (Kaya et al. 2009). Enhancement in chlorophyll pigments due to AMF is because of enhanced mineral uptake especially magnesium, an important component of chlorophyll molecule (Sheng et al. 2008). Higher chlorophyll contents in AMF-inoculated plants contribute to greater photosynthetic activity leading to maintain normal growth. Hence, it is clear that AMF inoculation enhances chlorophyll contents and also mitigates the negative impact of salinity to some extent. AMF symbiosis enhanced chloroplast functioning and photosynthetic ability of garlic plant under saline stress condition (Borde et al. 2010; Colla et al. 2008; Sheng et al. 2008).

Many researchers have reported that AMF inoculation could enhance the ability of plants to cope up with salinity stress (Talaat and Shawky 2011; Abdel-Fattah and Asrar 2012; Cekic et al. 2012). AMF colonization enhanced relative water content in *Zucchini* leaves (Colla et al. 2008), water potential and photosynthesis of maize plants (Sheng et al. 2008), and chlorophyll concentration in the leaves of several plant species, i.e., *Sesbania aegyptiaca*, *Sesbania grandiflora*, and *Lotus glaber* (Colla et al. 2008) under salinity stress condition. Generally, salinity stress has negative effect on AMF colonization; yet some reports have shown improved growth and productivity of mycorrhizal plants under saline conditions (Dudhane et al. 2011; Talaat and Shawky 2011; Cekic et al. 2012).

5.5 Salinity Tolerance by AMF Through Antioxidant Defense Response

Besides soil salinity, the increase in the content of salt in soil solution leads to imbalance in nutrients and ion. Due to this imbalance in ion and nutrients, the level of ROS generation in the plant increases considerably. Salinity hinders plant health and therefore to understand the various mechanisms that enable plant to overcome salt-induced stress and growth is essential. Plant cope up salt stress by increasing the production of some osmolytes and antioxidant enzymes which protect the plant cell from oxidative damage (Rai et al. 2011). Plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes in order to defend themselves against oxidants (Jiang and Zhang 2002; Núñez et al. 2003/2004). When pathogens attack the plant, plant reacts to the attack by activating its defense mechanism such as POD and CAT which are involved in cell wall strengthening or by their role as antioxidant (Mehdy 1994). Superoxide dismutase (SOD) is the basic antioxidant enzyme that converts superoxide to oxygen and hydrogen peroxide (H_2O_2) (Alscher et al. 2002). Besides SOD, CAT is also involved in scavenging H_2O_2 by decomposing and converting it into water and oxygen. Therefore, SOD

and CAT are treated as the main components which respond and regulate the antioxidant activities by controlling concentration O_2^- and H_2O_2 produced in the plant during stress (Van Breusegem et al. 2001).

Oxidative types of stresses are induced by salt stress in plant (Hajiboland et al. 2009). Antioxidant enzymes, being the best defense mechanism, help plant to fight against oxidative damage induced by stress. The production of ROS during various environmental stresses which also include salinity stress is considered as a major factor responsible for damaging the crop plant (Hernandez et al. 1995). The accumulation of ROS has serious impact on plants, especially the disturbance it causes in the metabolism of the plant through oxidative damage (Jiang and Zhang 2001). In order to reduce the damage caused by ROS, plants have their own protective mechanism which reduces the frequency of oxidative damage (Abdel Latif 2010). Detoxification of ROS is done by the enzymes which include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR) (Ahmad et al. 2008, 2010; Liu et al. 2014; Ahanger et al. 2014).

To reduce the adverse effect of stress, plant itself induces some antioxidant enzymes, but mycorrhizal association in plant helps to enhance host's antioxidant defense mechanism. These enzymes are continuously generated in mitochondria, peroxisomes, and cytoplasm of the plant.

Antioxidant defense system is positively associated with salt stress defense response of mycorrhizal colonized plants. It was also found that higher antioxidant enzyme activities in AMF plant can be correlated to plant growth improvement under salt stress (Alguacil et al. 2003; Zhong Qun et al. 2007).

Zhong Qun et al. (2007) reported that in tomato, the activities of SOD, CAT, and POD were observed to be higher in salinity-stressed AMF-inoculated plants as compared to control salt-stressed seedlings. Similar observations were reported in maize by Tang et al. (2009). Also, the increase in the activity of CAT resulted by inoculation of AMF has been reported in soybean (Ghorbanli et al. 2004). Under salinity stress condition, stomatal closure increases the consumption of the NADPH.

Under saline conditions, plant initiates antioxidative defense mechanism, to protect from harmful effect of ROS. In another study, low (50 mM) NaCl and moderate (100 mM) NaCl caused significant increase in shoot SOD activity in mycorrhizal plant as compared to non-mycorrhizal *Pennisetum glaucum* plant (Borde et al. 2011). Enhanced SOD activity in mycorrhizal plant could help in beneficial effect of mycorrhizal colonization (Borde et al. 2011). The increase in POD activity in mycorrhizal plant detoxifies the harmful effect of ROS (Borde et al. 2011). Whereas Catalase involves in decomposition of H_2O_2 in peroxisomes of mycorrhizal *Pennisetum glaucum* shoot under moderate and high salinity stress condition.

Different AMF fungal species showed varying extent of antioxidant enzyme activity in maize crop. The salinity stress-affected plant showed increase in SOD activity in root rather than shoot. Mycorrhizal plants showed higher SOD activity than non-mycorrhizal maize crop (Ruiz-Lozano et al. 2013). This increase in SOD activity in mycorrhizal maize crop helps to cope up with oxidative stress. In maize

plant, CAT activity is not evident in salt stress condition, suggesting that the AMF symbiosis does not affect the CAT activity under salt stress condition. (Ruiz-Lozano et al. 2013).

Under salt stress, the osmolytes contribute to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Ashraf and Foolad 2007). Compatible solutes are normally present in very small amount in plant when plant grows under normal condition. But, the concentration of these osmolytes increases rapidly after salt stress exposure. Accumulation of these osmolytes under salt stress varies with host plant as well as AMF species (Rabie and Almadini 2005). Accumulation of compatible solutes in the host plant is referred as positive physiological index under salt stress. These osmolytes help to maintain osmotic balance and membrane integrity and also this acts as a main reservoir of energy and nitrogen for use by plants under salt stress (Ashraf and Foolad 2007).

5.6 Salinity Tolerance by AMF Through Proline Accumulation

Proline is the most common osmolytes in plants, which plays an important role in increasing adaptability of plant under salinity stress conditions. It is one of the organic solutes synthesized by plant in response to drought and salinity stress and play an important role in maintaining osmotic adjustment of cell to ameliorate the salt stress effect. Proline is synthesized by two enzymes like pyroline-5 carboxylate synthase (P-5 Cs) and pyroline-5 carboxylate reductase (P-5 Cr) (Kishor et al. 1995). Proline is an important organic compound which participates in osmotic adjustment during abiotic stress conditions (Kishor et al. 1995).

AMF increase proline accumulation in plants subjected to drought stress (Smith and Read 2008). Proline is one of the osmolytes which accumulates in less salinity tolerance species, which modulates the salt stress through osmotic adjustment, plays a multiple role in plant stress tolerance (Yoshida et al. 1995), protects macromolecules during dehydration (Sanchez et al. 1998), and serves as a hydroxyl radical scavenger (Alia et al. 1995). Thus, AMF are considered to act as bio-ameliorators of saline soils (Singh et al. 1997). Sannazzaro et al. (2007) showed that *G. intraradices* inoculation in two genotype of *Lotus glaber* affect polyamine and Proline accumulation under salinity stress. Datta and Kulkarni (2014) showed increase in proline content in mycorrhiza-inoculated *Glycine max* and *Cyamopsis tetragonoloba legumes* under salinity stress conditions.

Some researchers have reported that enhanced concentration of osmolytes in mycorrhizal plants under salt stress (Feng et al. 2002; Al-Garni 2006; Sharifi et al. 2007; Garg and Manchanda 2009), no effort has been made to compare it with reference to protect ultra structural damage. In view of the defensive mechanism of the osmolytes on the ultrastructure of cells, it is assumed that AMF-mediated

increase in osmolytes concentrations might reduce ultrastructural damage of cells under salt stress. Higher concentration of osmolytes in mycorrhizal over non-mycorrhizal plants under salt stress has been reported in *Phragmites australis*, *Lotus glaber*, *Cajanus Cajan*, and *Z. mays* (Al-Garni 2006; Sannazzaro et al. 2007; Garg and Manchanda 2009; Sheng et al. 2011). These are the compatible solutes like free proline and glycine betaine which contribute to osmoregulation through maintaining the cell water content (Ahmad et al. 2008; Ahanger et al. 2014). AMF association with plant improved plant growth by enhancing physicochemical characteristics of rhizospheric soil (Asghari et al. 2005; Ahanger et al. 2014) and enhancing mobilization and uptake of several essential macro- and micronutrients in soil through modifying the root architecture (Ahanger et al. 2014; Hameed et al. 2014).

The proline accumulation is significantly higher in AMF inoculated garlic shoot than non-AMF shoot under low to moderate saline condition, whereas root proline accumulation was higher in mycorrhizal plants than non-mycorrhizal plants. But, overall more proline accumulation is observed in root than shoot because the roots are the primary sites for salinity stress condition (Borde et al. 2011). In Fenugreek plant, the non-mycorrhizal plant showed significantly higher proline accumulation than mycorrhizal plant under saline stress. The lower accumulation of proline in mycorrhizal fenugreek plant leads to reduced salt stress effect (Sannazzaro et al. 2007; Evelin et al. 2013). *Arachis hypogea* showed more proline accumulation in mycorrhizal plant than non-mycorrhizal plant under salt stress. Mycorrhizal *Arachis hypogea* plant adjusts better osmotic balance under stress condition than non-mycorrhizal plant (Al-Khaliel 2010). The alteration of higher proline accumulation under mycorrhization may be due to the enhancement in the activity of proline synthesizing enzymes and reduction in catabolizing ones or its restricted incorporation during protein synthesis (Ahmad et al. 2010). Increased proline accumulations due to AMF are in support by the findings of Shekoofeh et al. (2012) for *Ocimum basilicum*.

To conclude this chapter, salinity is considered to be among the most damaging stress faced by the plants when survival and productivity are in concern. The results conclude that the AMF alleviate the detrimental effect of salinity through improved plant growth by increasing the physiological activities in plants such as photosynthetic ability, relative water content, selective uptake of nutrient, and maintaining the ionic balance of plants. One of the salinity tolerance mechanisms triggered by AM inoculation is increased in the antioxidant enzyme activity of plants like POD, SOD, and CAT which scavenge the ROS and alleviate the salinity stress. Another mechanism of salinity tolerance by AMF colonization increased the non-antioxidative mechanism of plant mainly by accumulating the osmolytes such as Proline; this maintains the osmotic adjustment of plants under salinity stress condition. This cumulative effect increases the physiological performance and tolerance of the plants under salinity stress condition. For this reason, the application of AMF inoculum is more likely to give economical benefits when performed on high-valued crops.

Future studies need to be focused on detailed understanding of the key factors that can be used to overcome the problem of salinity and tolerance of salinity by inoculating the plants with AMF with reference to AMF species producing various antioxidants, proline accumulation, increasing P and K⁺ uptake, and increasing the plant growth through changing the physiological status of plants under salinity stress conditions. Study also need to be done for expression and characterization of certain stress-related genes, finding links and manipulation of various metabolic pathways that AMF fungi trigger to overcome the problem of salinity stress.

References

- Abd-Alla MH, Omar SA, Karanxha S (2000) The impact of pesticides on arbuscular mycorrhizal and nitrogen-fixing symbiosis in legumes. *Appl Soil Ecol* 14:191–200
- Abdel Latief AA (2010) Changes of antioxidative enzymes in salinity tolerance among different wheat cultivars. *Cereal Res Commun* 38:43–55
- Abdel-Fattah GM, Asrar AA (2012) Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil. *Acta Physiol Plant* 34:267–277
- Abdel-Fattah GM, Asrar AA, Al-Amri SM, Abdel-Salam EM (2014) Influence of arbuscular mycorrhiza and phosphorus fertilization on the gas exchange, growth and phosphatase activity of soybean (*Glycine max* L.) plants. *Photosynthetica* 52:581–588
- Adiku G, Renger M, Wessolek G, Facklam M, Hech-Bischoltz C (2001) Simulation of dry matter production and seed yield of common beans under varying soil water and salinity conditions. *Agric Water Manag* 47:55–68
- Ahanger MA, Tyagi SR, Wani MR, Ahmad P (2014) Drought tolerance: role of organic osmolytes, growth regulators, and mineral nutrients. In: Ahmad P, Wani MR (eds) [Physiological mechanisms and adaptation strategies in plants under changing environment](#), vol 1. Springer, New York, NY, pp 25–55
- Ahmad P, Gadgil K, Sharma S (2008) Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environ* 54:262–270
- Ahmad P, Jaleel CA, Sharma S (2010) Antioxidative defense system, lipid peroxidation, proline metabolizing enzymes and biochemical activity in two two *Morus alba* genotypes subjected to NaCl stress. *Russian J Plant Physiol* 57:509–517
- Ahmad P, Ozturk M, Sharma S, Gucel S (2014) Effect of sodium carbonate-induced salinity-alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (*Morus alba* L.) cultivars. *J Plant Interact* 9:460–467
- Al-Garni SMS (2006) Increasing NaCl-salt tolerance of a halophytic plant *Phragmites australis* by mycorrhizal symbiosis. *Am Eurasian J Agric Environ Sci* 1:119–126
- Alguacil MM, Hernández JA, Caravaca F, Portillo B, Roldán A (2003) Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforested in a degraded semi-arid soil. *Plant Physiol* 118:562–570
- Alia, Prasad KVSK, Saradhi PP (1995) Effect of zinc on free radicals and proline in *Brassica* and *Cajanus*. *Phytochemistry* 39:45–47
- Al-Karaki GN (2000) Growth and mineral acquisition by mycorrhizal tomato grown under salt stress. *Mycorrhiza* 10:51–54
- Al-Karaki GN (2006) Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. *Sci Hortic* 109:1–7
- Al-Khaliel AS (2010) Effect of salinity stress on mycorrhizal association and growth response of peanut infected by *Glomus mosseae*. *Plant Soil Environ* 56:318–324

- Alqarawi AA, Abd Allah EF, Hashem A (2014) Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. *J Plant Interact* 9:802–810
- Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* 53:1331–1341
- Aroca R, Porcel R, Ruiz-Lozano JM (2007) How does Arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytol* 173:808–816
- Aroca R, Ruiz-Lozano JM, Zamarreno AM, Paz JA, García-Mina JM, Pozo MJ, López-Ráez JA (2013) Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. *J Plant Physiol* 170:47–55
- Asghari HR, Marschner P, Smith SE, Smith FA (2005) Growth response of *Atriplex nummularia* to inoculation with arbuscular mycorrhizal fungi at different salinity levels. *Plant Soil* 273:245–256
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:207–216
- Bago B, Pfeffer PE, Shachar-Hill Y (2000) Carbon metabolism and transport in arbuscular mycorrhiza. *Plant Physiol* 124:949–958
- Berta G, Fusconi A, Trotta A, Scannerini S (1990) Morphogenetic modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum* L. *New Phytol* 114:207–215
- Borde MY, Dudhane MP, Jite PK (2010) AM fungi influences the photosynthetic activity, growth and antioxidant enzymes in *Allium sativum* L. under salinity condition. *Notulae Scientia Biologicae* 2:64–71
- Borde MY, Dudhane MP, Jite PK (2011) Growth photosynthetic activity and antioxidant responses of mycorrhizal and non-mycorrhizal bajra (*Pennisetum glaucum*) crop under salinity stress condition. *Crop Prot* 30:265–271
- Bothe H (2012) Arbuscular mycorrhiza and salt tolerance of plants. *Symbiosis* 58:7–16
- Cantrell IC, Linderman RG (2001) Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant Soil* 233:269–281
- Cekic FO, Unyayar S, Ortas I (2012) Effects of arbuscular mycorrhizal inoculation on biochemical parameters in *Capsicum annuum* grown under long term salt stress. *Turk J Bot* 36:63–72
- Colla G, Roupheal Y, Cardarelli M, Tullio M, Rivera CM, Rea E (2008) Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biol Fertil Soils* 44:501–509
- Datta P, Kulkarni M (2014) Arbuscular mycorrhizal colonization enhances biochemical status in and mitigates adverse salt effect on two legumes. *Notulae Scientia Biologicae* 6:381–393
- Doganlar ZB, Demir K, Basak H, Gul I (2010) Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. *Afr J Agric Res* 5:2056–2065
- Dudhane MP, Borde MY, Jite PK (2011) Effect of arbuscular mycorrhizal fungi on growth and antioxidant activity in *Gmelina arborea* Roxb. under salt stress condition. *Notulae Scientia Biologicae* 3:71–78
- Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann Bot* 104:1263–1280
- Evelin H, Giri B, Kapoor R (2012) Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. *Mycorrhiza* 22:203–217
- Evelin H, Giri B, Kapoor R (2013) Ultra structural evidence for AMF mediated salt stress mitigation in *Trigonella foenum-graecum*. *Mycorrhiza* 23:71–86
- Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z (2002) Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* 12:185–190

- Garg N, Manchanda G (2009) Role of arbuscular mycorrhizae in the alleviation of ionic, osmotic and oxidative stresses induced by Mycorrhiza salinity in *Cajanus cajan* (L.) Millsp. (pigeonpea). *J Agron Crop Sci* 195:110–123
- Ghazi J, Al-Karaki GN (2001) Mycorrhizal influence on fruit yield and mineral content of tomato grown under salt stress. *J Plant Nutr* 24:1311–1323
- Ghorbanli H, Ebrahimzadeh M, Sharifi M (2004) Effects of NaCl and mycorrhizal fungi on antioxidative enzymes in soybean. *Biol Plant* 48:575–581
- Giri B, Mukerji KG (2004) Mycorrhizal inoculants alleviate salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. *Mycorrhiza* 14:307–312
- Giri B, Kapoor R, Mukerji KG (2003) Influence of Arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of *Acacia auriculiformis*. *Biol Fertil Soils* 38:170–175
- Giri B, Kapoor R, Mukerji KG (2007) Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. *Microb Ecol* 54:753–760
- Gupta ML, Khaliq A, Pandey R, Shukla RS, Singh HK, Kumar S (2000) Vesicular–arbuscular mycorrhizal fungi associated with *Ocimum* spp. *J Herbs Spices Med Plants* 7:57–63
- Hajiboland R, Joudmand A, Fotouhi K (2009) The K/Na replacement and function of antioxidant defense system in sugar beet (*Beta vulgaris* L.) cultivars. *Acta Agric Scand Sect B-Soil Plant Sci* 59:246–259
- Hajiboland R, Aliasgharzadeh S, Laiegh F, Poschenrieder C (2010) Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil* 331:313–327
- Hameed A, Egamberdieva D, Abd Allah EF, Hashem A, Kumar A, Ahmad P (2014) Salinity stress and arbuscular mycorrhizal symbiosis in plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses, vol 1. Springer, New York, pp 139–159
- Hammer EC, Nasr H, Pallon J, Olsson PA, Wallander H (2011) Elemental composition of arbuscular mycorrhizal fungi at high salinity. *Mycorrhiza* 21:117–129
- Hashem A, Abd-Allah EF, Alqarawi AA, ElDidamony G, Alwhibi MS, Egamberdieva D, Ahmad P (2014b) Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. *Pak J Bot* 46:2003–2013
- He Z, He C, Zhang Z, Zou Z, Wang H (2007) Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. *Colloids Surf B: Biointerfaces* 59:128–133
- Hernandez JA, Olmos E, Corpas FJ, Sevilla F, Delrio LA (1995) Salt induced oxidative stress in chloroplast of pea plants. *Plant Sci* 105:151–167
- Hu YC, Schmidhalter U (2005) Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J Plant Nutr Soil Sci* 168:541–549
- Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM (2008) Influence of salinity on the *in vitro* development of *Glomus intraradices* and on the *in vivo* physiological and molecular responses of mycorrhizal lettuce plants. *Microb Ecol* 55:45–53
- Jiang M, Zhang J (2001) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *J Exp Bot* 53:2401–2410
- Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize-leaves. *J Exp Bot* 53:2401–2410
- Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL, Cullu MA (2009) The influence of arbuscular mycorrhizal colonization on key growth parameters and fruit yield of pepper plants grown at high salinity. *Sci Hortic* 121:1–6
- Kishor, PBK, Hong, Z, Miao, G.H, Hu, CAA, Verma, D.P.S. (1995). Over expression of $\Delta 1$ -pyrroline-5-carboxylate synthetase increase proline production and confers osmotolerance in transgenic plants. *Plant Physiol*, 108:1387–1394

- Kohler J, Hernández JA, Caravaca F, Roldan A (2009) Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environ Exp Bot* 65:245–252
- Liu L, Sun H, Chen J, Zhang Y, Li D, Li C (2014) Effects of cadmium (Cd) on seedling growth traits and photosynthesis parameters in cotton. *Plant Omics J* 7:284–290
- Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. *Arch Biochem Biophys* 444:139–158
- Mehdy MC (1994) Active oxygen species in plant defense against pathogens. *Plant Physiol* 105:467–472
- Munns R, Gilliam M (2015) Salinity tolerance of crops—what is the cost? *New Phytol* 208:668–673
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681
- Núñez M, Mazzafera P, Mazorra LM, Siqueira WJ, Zullo MAT (2003/2004) Influence of brassinosteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. *Biologia Plantarum* 47:67–70
- Parida SK, Das AB (2005) Salt tolerance and salinity effects on plants. *Ecotoxicol Environ Saf* 60:324–349
- Patel D, Saraf M (2013) Influence of soil ameliorants and microflora on induction of antioxidant enzymes and growth promotion of *Jatropha curcas* L. under saline condition. *Eur J Soil Biol* 55:47–54
- Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi: a review. *Agron Sustain Dev* 32:181–200
- Prasad R, Bholra D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) *Mycorrhiza*. Springer, Cham, pp 1–7
- Rabie GH (2005) Influence of arbuscular mycorrhizal fungi and kinetin on the response of mungbean plants to irrigation with seawater. *Mycorrhiza* 15:225–230
- Rabie GH, Almadini AM (2005) Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. *Afr J Biotechnol* 4:210–220
- Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan AK (2011) Developing stress tolerant plants through in vitro selection – an overview of the recent progress. *Environ Exp Bot* 71:89–98
- Ramolija PJ, Patel HM, Pandey AN (2004) Effect of salinization of soil on growth and macro- and micronutrient accumulation in seedlings of *Acacia catechu* (Mimosaceae). *Ann Appl Biol* 144:321–332
- Ramos AC, Facanha AR, Palma LM, Okorokov LA, Cruz ZMA, Silva AG (2011) An outlook on ion signaling and ionome of mycorrhizal symbiosis. *Braz J Plant Physiol* 23:79–89
- Rao AV, Tak R (2002) Growth of different tree species and their nutrient uptake in limestone mine spoil as influenced by arbuscular mycorrhizal (AM) fungi in India arid zone. *J Arid Environ* 51:113–119
- Rasool S, Ahmad A, Siddiqi TO, Ahmad P (2013) Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiol Plant* 35:1039–1050
- Roberts JKM, Linker CS, Beoit AG, Jardetzky O, Nieman RH (1984) Salt stimulation of phosphate uptake in maize root tips studied by ³¹P nuclear magnetic resonance. *Plant Physiol* 75:947–950
- Ruiz-Lozano JM, Azcon R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. *Physiol Plant* 95:472–478
- Ruiz-Lozano JM, Collados C, Barea JM, Azcon R (2001) Arbuscular mycorrhizal symbiosis can alleviate drought induced nodule senescence in soybean plants. *Plant Physiol* 82:346–350

- Ruiz-Lozano JM, Estrada B, Aroca R, Barea JM (2013) Native arbuscular mycorrhizal fungi isolated from a saline habitat improved maize antioxidant systems and plant tolerance to salinity. *Plant Sci* 201–202:42–51. doi:10.1016/j.plantsci.2012.11.009
- Sanchez FJ, Manzanares M, De Andres EF, Tenorio JL, Ayerbe L (1998) Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. *Field Crop Res* 59:225–235
- Sannazzaro AI, Echeverria M, Alberto EO, Ruiz OA, Menendez AB (2007) Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. *Plant Physiol Biochem* 45:39–46
- Schubler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res* 105:1413–1421
- Serrano R, Rodriguez-Navarro A (2001) Ion homeostasis during salt stress in plants. *Curr Opin Cell Biol* 13:399–404
- Sharifi M, Ghorbanli M, Ebrahimzadeh H (2007) Improved growth of salinity-stressed soybean after inoculation with pre-treated mycorrhizal fungi. *J Plant Physiol* 164:1144–1151
- Shekoofeh E, Sepideh H, Roya R (2012) Role of mycorrhizal fungi and salicylic acid in salinity tolerance of *Ocimum basilicum* resistance to salinity. *Afr J Biotechnol* 11:2223–2235
- Sheng M, Tang M, Chan H, Yang B, Zhang F, Huang Y (2008) Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* 18:287–296
- Sheng M, Tang M, Zhang F, Huang Y (2011) Influence of arbuscular mycorrhiza on organic solutes in maize leaves under salt stress. *Mycorrhiza* 21:423–430
- Shi LX, Guo JX (2006) Changes in photosynthetic and growth characteristics of *Leymus chinensis* community along the retrogression on the Songnen grassland in northeastern China. *Photosynthetica* 44:542–547
- Shi DC, Li YM, Yang GH, Li YD, Zhao KF (2002) A simulation of salt and alkali mixed ecological conditions and analysis of their stress factors in the seedlings of *Aneurolepidium chinense*. *Acta Ecol Sin* 22:1323–1332
- Shokri S, Maadi B (2009) Effects of arbuscular mycorrhizal fungus on the mineral nutrition and yield of *Trifolium alexandrinum* plants under salinity stress. *J Agron* 8:79–83
- Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *J Ecol* 97:1274–1280
- Singh RP, Choudhary A, Gulati A, Dahiya HC, Jaiwal PK, Sengar RS (1997) Response of plants to salinity in interaction with other abiotic and factors. In: Jaiwal PK, Singh RP, Gulati A (eds) *Strategies for improving salt tolerance in higher plants*. Science Publishers, Enfield, NH, pp 25–39
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic Press, London
- Talaat NB, Shawky BT (2011) Influence of arbuscular mycorrhizae on yield, nutrients, organic solutes, and antioxidant enzymes of two wheat cultivars under salt stress. *J Plant Nutr Soil Sci* 174:283–291
- Talaat NB, Shawky BT (2012) 24-Epibrassinolide ameliorates the saline stress and improves the productivity of wheat (*Triticum aestivum* L.) *Environ Exp Bot* 82:80–88
- Tang M, Chen H, Huang JC, Tian ZQ (2009) AM fungi effects on the growth and physiology of *Zea mays* seedlings under diesel stress. *Soil Biol Biochem* 41:936–940
- Tomar NS, Agarwal RM (2013) Influence of treatment of *Jatropha curcas* L. leachates and potassium on growth and phytochemical constituents of wheat (*Triticum aestivum* L.) *Am J Plant Sci* 4:1134–1150
- Van Breusegem F, Vranová E, Dat JF, Inzé D (2001) The role of active oxygen species in plant signal transduction. *Plant Sci* 161:405–414
- Wu QS, Zou YN, He XH (2010) Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. *Acta Physiol Plant* 32:297–304

- Wu QS, Zou YN, Abd Allah EF (2014) Mycorrhizal association and ROS in plants. In: Ahmad P (ed) Oxidative damage to plants. Elsevier, New York, pp 453–475
- Yamato M, Ikeda S, Iwase K (2008) Community of arbuscular mycorrhizal fungi in coastal vegetation on Okinawa Island and effect of the isolated fungi on growth of sorghum under salt-treated conditions. *Mycorrhiza* 18:241–249
- Yano-Melo AM, Saggin OJ, Maia LC (2003) Tolerance of mycorrhized banana (*Musa* sp. cv. Pacovan) plantlets to saline stress. *Agric Ecosyst Environ* 95:343–348
- Yoshiba Y, Kiyosue T, Katagiri T, Ueda H, Mizoguchi T, Yamaguchishinozaki K, Wada K, Harada Y, Shinozaki K (1995) Correlation between the induction of a gene for delta (1)-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. *Plant J* 7:751–760
- Zhong Qun H, Chao Xing H, Zhibin Z, Zhirong Z, Huai Song W (2007) Changes in antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. *Colloids Surf B: Biointerfaces* 59:128–133
- Zuccarini P, Okurowska P (2008) Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. *J Plant Nutr* 31:497–513

Chapter 6

Arbuscular Mycorrhizal Technology Based on Ecosystem Services Rendered by Native Flora for Improving Phosphorus Nutrition of Upland Rice: Status and Prospect

Dipankar Maiti, Neha Nancy Toppo, Mukesh Nitin, and Binit Kumar

Abstract Upland ecology is predominantly rainfed and drought prone having nutrient-poor, well-drained, acidic soils. Direct-seeded rice (*Oryza sativa* L.) is the major crop beside maize (*Zea mays* L.), pulses and oilseeds in this ecology. Small portion of uplands with assured irrigation is also grown with vegetables. Nearly one-sixth of world's rice land is under uplands (about 20 million ha) of which (upland rice area) almost two-thirds is in Asia (IRRI 1975; Gupta and O'Toole 1986). Upland farmers, particularly in Asia, are generally resource poor having small land holdings, and majority of them practice subsistence farming. Despite its (uplands) disadvantaged natural conditions for field crops leading to poor productivity, soil microbial health is comparatively less disturbed due to minimum use of modern agrochemicals. This makes the ecology suitable for manipulating beneficial soil microbial resources in favour of crop production. Poor phosphorus (P) acquisition by crops is one major constraint of this ecology. On the other hand, the aerobic soil conditions support arbuscular mycorrhizal (AM) activities which are known to facilitate P acquisition in associated plants. The present article deals with avenues of harnessing ecosystem services rendered by AM fungi (AMF) for improving P nutrition of upland rice under rice-based cropping systems.

6.1 Introduction

Sustainable agricultural systems are fast gaining global significance in view of the growing demand for ecologically sound and economically viable cultivation methods that reduce the pressure on the environment due to modern agricultural practices. Excessive application of agrochemicals intended for maximizing yield is

D. Maiti (✉) • N.N. Toppo • M. Nitin • B. Kumar
Central Rainfed Upland Rice Research Station, ICAR-National Rice Research Institute
(formerly Central Rice Research Institute), PB 48, Hazaribag 825 301, Jharkhand, India
e-mail: dipanlar_maiti@live.in

detrimental in the long run both in terms of degradation of environment and negative impacts on sustainable productivity issues. Judicious integration of microbial inoculant application in the forms of bio-fertilizers and biopesticides with that of synthetic chemicals, in the cultivation systems, is the only route to mitigate such adverse impact on the environment leading to harnessing optimum productivity on long-term basis (sustainable agriculture).

Arbuscular mycorrhiza fungi (AMF) are ubiquitous soil inhabitants, forming symbiosis with about 83% of dicotyledonous and 79% of monocotyledonous plants (Wilcox 1991) including most of the agriculturally and horticulturally important crop plants (Smith and Read 1997; Harrier and Watson 2003; Goltapeh et al. 2008; Prasad et al. 2017). AMF benefit host plant principally by increasing uptake of relatively immobile nutrients like phosphorus (P) (Bagyaraj et al. 2015). Phosphate ions (Pi) remain untrapped by plant unless being intercepted through root system, unlike relatively mobile nutrients like nitrogen (N) which becomes available to plant by means of mass flow. Extra-radical mycelial (ERM) network of AMF extends in the soil beyond the phosphate depletion zone (Marshner 1995; Smith and Read 1997; Johansen et al. 1993; Li et al. 1991a) that quickly develops around roots due to plant uptake of the mobile P present in the soluble/labile P pool. The new pool of labile P (adsorbed on soil particles) beyond P depletion zone is intercepted by an increase in the absorbing area via the external hyphae (ERM) of AMF connected to colonized plant roots and help in acquisition of this P fraction from soil by plant. In exchange, AMF obtain carbon (C) from the colonized plant. AMF colonization also provides additional benefits to the host including (1) improved drought resistance through enhanced tolerance of water stress (Abdelmoneim et al. 2014; Maiti et al. 2009a; Davies et al. 2002; Auge et al. 1994), (2) increased resistance to soil borne diseases (Whipps 2004; Huang et al. 2003; Lingua et al. 2002), (3) increased tolerance to salinity (Salim et al. 2013; Mohammad et al. 2003; Feng et al. 2002) and (4) metal toxicity (Lin et al. 2007; Hildebrandt et al. 2007; Diaz et al. 1996). AMF have also been noted to increase uptake of macronutrients other than phosphorus (P) including nitrogen (N), potassium (K) and also certain micronutrients like zinc (Zn), magnesium (Mg), copper (Cu), iron (Fe) and manganese (Mn) (Fattah 2013; Gosling et al. 2006; Vosatka et al. 2006; Smith and Read 1997; Li et al. 1991b). There is also emerging evidence that AMF reduce nutrient loss from soils by enlarging nutrient interception zone and preventing nutrient loss after rain-induced leaching events (Cavagnaro et al. 2015). Beside these, AMF have been shown to play an important role in maintaining soil aggregate stability (Srimathi et al. 2014; Ambriz et al. 2010; Degens et al. 1996; Tisdall 1991) by building up macroporous structure of soil that allows penetration of water and air and prevents soil erosion (Miller and Jastrow 1992). AMF produce an extracellular glycoprotein called glomalin, which supports hyphae to stick to soil, promoting formation of stable soil aggregates (Wright and Upadhyaya 1996). Thus, AMF are used in soil/land reclamation and revegetation (Wu et al. 2002; Requena et al. 2001; Miller and Jastrow 1992) and bioremediation and help mankind by promoting supply of protective (antioxidants) nutrient components to human beings through agricultural products

(Gianinazzi et al. 2010). In addition, AMF provide ecological advantages by influencing microbial and chemical environment of the mycorrhizosphere in favour of plant growth (Johansson et al. 2004).

A sustainable agricultural system essentially involves natural processes to achieve adequate levels of productivity and food quality while minimizing environmental pollution (Siddiqui and Pichtel 2008; Prasad et al. 2015). This includes minimum use of soluble mineral fertilizers and limiting the usage of synthetic pesticides crucial for crop protection against pests and diseases (Gosling et al. 2006). AMF can compensate for lower inputs of P fertilizers provided a high species diversity and an effective, active AMF community is encouraged and maintained in the soil via sound management practices so as to maximize benefits from AM association without conflicting with other beneficial farm management/agricultural practices. Therefore, the biological management of the key issue of poor P acquisition, through AMF technology, has gained considerable importance so as to accrue benefits (1) via the native or indigenous mycorrhizal fungi or (2) by the application of commercial inoculums containing exotic/non-native isolates. Both approaches have certain advantages and disadvantages. Under ecologically sound soil system with least microbial disturbances which (microbial disturbances) occur as a result of intensive agriculture, maintaining microbial diversity almost intact with efficient strains, exploitation of native population is more advantageous because:

- (1) It is very much cost-effective, particularly under rainfed agroecosystems with lower cropping intensity where inoculated population is crashed down due to long fallow disorder (Thompson 1987) and off-season soil desiccation (Maiti et al. 1996) necessitating repeated inoculum application
- (2) Native population are more adapted to the soil system ensuring minimum rejection by resident population which is usually encountered by exotic strains/species
- (3) It warrants no ecological threat of unintentional introduction of undesirable contaminants to the ecology (Schwartz et al. 2006)

An introduced inoculum with exotic microbial source may even depress yield if the native AMF population is effective (Kahiluoto and Vestberg 1998; Izaguirre-Mayoral et al. 2000; Klironomos 2003). Commercial AMF inoculants containing efficient exotic strains are, however, more effective for plantation crops and reclamation of degraded lands.

Upland ecology, predominantly grown with direct-seeded rice as major crop, is constrained by poor P acquisition under drought-prone condition (Fageria et al. 1982). However, aerobic soil conditions favour AM activities which facilitate P acquisition by colonized plants. Further, the low-input agriculture, practiced by the resource poor upland farmers, allows less disturbance of soil microbial community in terms of both population and diversity including that of AMF (Toppo et al. 2013). This situation provides ample scope for manipulating AM activities to improve P nutrition of upland rice and associated crops. Moreover, uneven distribution of monsoon rain, as a consequence of climate change, in recent years, has led to

unprecedented drought spells which further accentuated poor P nutrition under rainfed agroecosystems. Long-term experiment (1999–2009) data showed that native AM-aided P use efficiency of upland rice was higher in drought years with below-average rainfall over that of normal rainfall years (Maiti et al. 2013). This further justifies importance of AM technology under rainfed ecology. Possible avenues of exploiting native AMF flora in favour of improved P nutrition of crops under rainfed ecology with special reference to direct-seeded upland rice have been discussed in the present review.

6.2 Native AMF-Based Technology

Continuous attempts are ongoing worldwide to trap renewable ecosystem services rendered by vast and diverse groups of beneficial microbes harbouring in the environment. Majority of them are having niche in soil, the essential medium for agriculture. Unidirectional focus on gearing up productivity, to satisfy need of the time, during the last decade, through use of synthetic agricultural chemicals has deteriorated soil microbial health posing threat to sustainability of higher productivity. This situation has prompted the agriculturists, in recent time, to think of judicious integration of synthetic agrochemicals' use and trapping natural ecosystem services. Ecologies like uplands in India are less disturbed in terms of soil microbial diversity and, hence, more suitable for exploiting native beneficial microbes through practice of such 'microbe-supportive' crop culture methods. With this presumption, several potential crop components of upland rice and rice-based cropping systems of uplands were evaluated to identify most suitable native AMF-supportive components. The identified components were validated through on-farm trails in farmers' fields in participatory mode and were integrated to develop native AM technology for upland rice with in-built, eco-friendly native AMF-aided higher P use efficiency. The crop culture options evaluated were (1) cultural management, (2) rice-based cropping systems/rotations and (3) development of farmers' friendly method of AMF inoculum production of native origin and application.

6.2.1 Cultural Management Options

Among several, two cultural management options (agro-practices), viz. tillage (Jasper et al. 1991) and P application dose (Habte and Manjunath 1987), are having higher influence on native AMF activities in soil. Based on these observations, scientists attempted to manipulate these two agro-practices in favour of AM without compromising their direct effects on productivity.

Optimization of Tillage Schedule for Upland Rice Off-season tillage is agronomic recommendation, especially in uplands, for reducing weed and soilborne pest infestation. Repeated off-season tillage (OST), however, imposes soil disturbance-induced (SDI) deleterious effects on established mycelia network of AMF in soil-reducing colonization in subsequent crops (Jasper et al. 1991; McGonigle and Millar 1993a). Uplands in Asia are vulnerable to this effect due to lack of judicial planning or scheduling of OST which is normally done by upland farmers whenever off-season rain is received to attain proper soil moisture for tillage, using bullock or tractor drawn plough. Farmers also have very short window of getting soil conditions favourable for OST under this rainfed ecology. In such situation where OST cannot be totally ignored and also imposes deleterious effects on mycorrhizal activities in soil, efforts were made to optimize OST schedule under this ecology to minimize SDI effects. The initial results obtained under fixed plot experimentation in India revealed that a minimum space of 13 weeks between two OST would minimize SDI effects with most suitable OST schedule of one after harvest (rice) followed by one summer tillage (Maiti et al. 2011a).

Optimization of P Application Dose for Upland Rice High soil P availability limits AMF activities in soil (Habte and Manjunath 1987) and AMF-mediated P acquisition (Richardson et al. 2011) because it (high soil P) reduces formation of AMF-crop symbiosis mainly by lowering (1) growth rate of infection units, (2) production of secondary external hyphae, (3) spore germination and (4) effectiveness of AMF inocula (Kahiluoto et al. 2000, 2001). High P concentration in plant root, at the same time, reduces colonization due to reduced root membrane permeability resulting in decreased loss of AMF favouring metabolites (Graham et al. 1981; Smith and Read 1997; Vierheilig 2004). Under abundant soil P, direct uptake pathway is preferred by plant (Balzergue et al. 2011; Nagy et al. 2009), and reduced AMF colonization is observed (Sally et al. 2011). This necessitated optimization of P fertilizer dose under AMF inoculation to improve AMF efficacy and maintaining P economy without compromising productivity. Enhanced benefits through optimizing soil P were demonstrated in various crops (Habte and Manjunath 1987; Shukla et al. 2011). For upland rice under AMF-supportive rice-based crop rotation and AMF inoculation, about 33% reduction of recommended P dose was worked out to be optimum to achieve higher grain yield (+30.4%) and P economy to the tune of savings of about 10 kg P_2O_5 /ha (Maiti and Barnwal 2012).

6.2.2 AMF-Supportive Cropping System and Rotation Options

Soils used for agricultural productivity usually have low AMF diversity (Menendez et al. 2001; Verbruggen and Kiers 2010), distribution (Fontenla et al. 2001) and population compared to natural ecosystems. The reasons are adverse effects of agricultural operations (Oehl et al. 2005) like soil disturbance-induced (SDI)

deleterious effects of tillage on established mycelial (AMF) network (Jasper et al. 1991; McGonigle and Millar 1993b) and limiting effects of application of fertilizer(s), particularly P, on AMF activity (Kahiluoto et al. 2000, 2001). This diminishes prospects of reaping benefits from these soil fungi as they need to re-establish their network and activities in soil. Such effects were accentuated under intensive agriculture system over recent past. In Asian and African countries, the problem has further exacerbated under monocropping systems (Sharma et al. 2010). Monocropping tends to select those AMF species which grow and sporulate rapidly. These species offer the least benefit to the plant as they divert more resources to their own growth and reproduction. Moreover, there may be further reduction in the AMF population and activities in soil during no-crop period (Harinikumar and Bagyaraj 2005) or long fallow leading to 'long fallow disorder' (Thompson 1987). Even land cover with non-host (AMF) crop has been demonstrated to be better than long fallow in terms of AMF colonization to subsequent crop (Ocampo and Hayman 1981) and spore population in soil (Kruckelmann 1975). This can be attributed to the previous findings that AM fungal hyphae can make some hyphal growth around the roots of non-host plants without colonizing the roots due to the absence of signals from non-host roots required by AM fungi for successful colonization (Ocampo et al. 1980). Such roots surrounded by AM hyphal growth are more efficient in colonizing host plants than chlamydo spores or other inoculum sources. Such unfavourable factors (monocropping and fallow disorder) can be addressed by introducing multi-crop approach with AMF-supportive cropping systems and crop rotations (Ocampo and Hayman 1981). Introduction of AMF-supportive cropping systems including AMF host plants, specific to ecology, would further support healthy soil-AMF environment. Enhanced P nutrition of upland rice under AM-supportive cropping systems and rotations has been elaborately reviewed and discussed by Maiti (2011). The options described in the review included increased AMF colonization and concomitant-enhanced P uptake in (1) direct-seeded upland rice under AM-supportive rice-based cropping systems (Rana et al. 2002) and rotations (Maiti et al. 2006, 2012) and (2) transplanted rice grown in rainfed drought-prone medium lands from seedlings raised in plots pre-cropped with AM-susceptible fodder grasses (Maiti et al. 2008).

6.2.3 Developing Farmers' Friendly Method of AMF Inoculum Production of Native Origin and Application

Commercial inoculums of efficient isolates/strains of AMF are available in market which is useful for plantation, horticultural and cash crops. The use of commercial AMF inoculum, however, has some disadvantages: (1) there is greater propensity of natural soil microflora including native AMF to reject intruders (commercial inoculums), and (2) such inoculums may have possible negative ecological consequences in terms of invasive species introduction as unintended contaminants

(Schwartz et al. 2006). Such constraints of commercial inoculums are more acute in upland ecology where native AMF population of soil has not been much damaged by biologically unsound processes including modern, chemical-based agricultural practices. Moreover, under predominantly monocropped (rice/maize/pulses) upland ecology, soil population crashes under strong ecological stresses like long duration post-crop fallowing, soil desiccation, etc., necessitating frequent use of inoculums which is not cost-effective for low-value field crops like rice. Introduction of exotic species/strain (AMF) through application of commercial inoculums may even depress yield if the native AMF population is effective (Klironomos 2003; Izaguirre-Mayoral et al. 2000; Kahiluoto and Vestberg 1999). Khaliq and Sanders (2000) measured a small (3–4%) reduction in the yield of barley in response to inoculation with a single AMF species. So, AMF inoculums developed from native sources consisting of consortium of AMF species are considered to be more efficient (Oliveira et al. 2005), cost-effective and adaptable to the target ecology. The native biological potential of soils, including that of AMF, is expected to be least disturbed under situations of low input system cultivation as are normally practiced in poorly productive upland ecology, particularly under rice cultivation. There is evidence of diverse (Maiti et al. 1995; Toppo and Maiti 2011) and effective (Maiti et al. 2012) AMF flora in upland soils under rice cultivation.

On-farm technique of inoculum production with AMF (Douds et al. 2005, 2006; Gaur et al. 2000; Gaur 1997; Sieverdin 1991) of native origin (Gaur and Adholeya 2002) has been widely explored and extensively reviewed by Marleen et al. (2011) and Maiti (2011). Based on the knowledge and technology generated, a production protocol of AMF inoculum using native AMF, specifically suitable to upland rice, was developed by Maiti et al. (2009b), and its efficacy in terms of improving P nutrition of rice has been confirmed (Maiti et al. 2011b). The protocol allows the farmers to produce cheaper, soil-based high-volume inoculums of their own through multiplying nucleus inoculums (NI) (starter inoculums) within their farm system. The nucleus inoculums consist of a consortium of spores (AMF) indigenous to the site of application and not always identified to the species level (Gaur and Adholeya 2002). The NI is multiplied on *Sorghum* (*Sorghum bicolor*) roots grown in micro-plots, presterilized (partially) by soil solarization using transparent, thin (1–2 mm) low-density polyethylene (LDPE) film mulching during peak summer months to produce mass inoculums (MI). MI essentially consist of external hyphae (AMF), infected roots, spores and soil (15 cm depth from the solarized plots). The protocol scheme involves production of NI under controlled condition by institutions, supplying to the farmers who can multiply NI in their farm after training and produce mass inoculum (MI). Integration of native AM-supportive crop culture components (AM-supportive rice-based cropping systems and application of AM-inoculum developed from native sources) showed to produce additive effects on native AMF activity, plant growth promotion, P uptake and grain yield of upland rice under rainfed ecology (Maiti et al. 2011b).

Use of Multifunctional Microbial Consortium AMF inoculum has been tried in combination with several other beneficial microorganisms to achieve possible

additive or synergistic effects. Benefits of co-inoculation of AMF with (1) N-fixing non-symbiotic bacteria like *Azotobacter chroococcum* (Umakant and Bagyaraj 1998) and *Azospirillum* (Rangarajan and Santhanakrishnan 1995) in mulberry, (2) plant growth-promoting rhizobacteria (PGPR; *Pseudomonas fluorescens*) in *Morus alba* (Rangarajan and Santhanakrishnan 1995), (3) phosphate-solubilizing bacteria (PSB) like *Bacillus licheniformis* in upland rice and (4) PSB (*B. megaterium*) and N-fixer (*Azotobacter*) in mulberry (Baqual and Das 2006) were demonstrated. Co-inoculation of different beneficial microbes has proved to be better because it supports plant growth through various ways and also supports each other's growth.

6.3 Innovative Option of Exploiting Native AMF: Use of AM-Responsive Crop Varieties

Possible avenues of exploiting native AMF by agronomic manipulation (adoption of AM-supportive crop culture components) and use of AMF inoculum of native origin have been discussed in the previous chapters. In recent years, researchers are emphasizing on another innovative approach of exploiting native AMF flora through taking advantage of potential of plant species (host) to harness ecosystem services rendered by native AMF. This could be achieved by genetic manipulation of crop varieties for enhanced AM response. The agronomic and genetic manipulations for enhanced mycorrhizal nutrient acquisition and response are mutually inclusive and in combination could exploit (native AMF biodiversity in soil), to the full extent. Mycorrhiza responsiveness has been defined as difference in growth response between mycorrhizal and non-mycorrhizal plants under a given environment (Janos 1988). Siqueira and Saggin (2001) clarified that responsiveness relates to its internal nutrient demand in relation to growth rate under a given environment. Smith and Smith (2011) quantified 'mycorrhizal responsiveness (MR)' in terms of change in plant biomass that results from the symbiosis.

The approach of genetic manipulation (crop) is based on twin attributes of AM symbiosis—lack of host specificity of AMF and variation in AM responsiveness among genotypes of plants. The native AMF flora can be exploited best, provided that (1) the native population remains undisturbed and (2) the crop varieties are highly responsive to AMF both for colonization and P acquisition. Presences of diverse and effective AMF flora, particularly in less biologically disturbed ecology, like uplands, have been confirmed (Maiti et al. 1995). On the second aspect, there is sufficient evidence that plant response to arbuscular mycorrhiza is a variable trait (Smith and Read 1997) and there is scope to exploit the variability to select/breed high mycorrhiza-responsive crop varieties for exploiting the biological potentials of AM in nutrition management of crop plants. AM responsiveness of plants operates at two levels—(1) root colonization and (2) physiological response (including P acquisition) following colonization. Host genotype variations in the extent of

colonization are known in many crop species, including members of the Gramineae (Barker et al. 2002) where high colonization of a genotype does not necessarily translate to high physiological response of the plant (Ravenskov and Jakobsen 1995). Plant species or varieties are reckoned to vary in functional compatibility with AMF where P uptake of one species or a variety may improve more than that of another following colonization by the same AMF species (Burleigh and Bechmann 2002). There have been a few attempts to dissect such functional compatibility or response variation of crop plants genetically, so as to identify the genes that determine/control 'enhanced AM responsiveness'. Such identification holds prospect for use and application in breeding crop plants for high AM responsiveness (Barker et al. 2002), especially for systems of low input cultivation, like the upland rice. The modern molecular tools have widened the scope of identification of such hidden or obscure genes with less time and cost than the conventional methods.

Insights on genetic analysis of AMF symbiosis of crop plants have come from molecular analysis of mycorrhiza-defective mutant of legume hosts—garden pea, vetch, clover and *Lotus japonicus* as model plants. These studies have helped identifying more than 20 loci in different legume species (Marsch and Schultze 2001) which control development of symbiosis, particularly the stages of penetration and post-penetration colonization by the non-host-specific AM fungi (Barker et al. 2002). Characteristically, some of these loci (17 identified till now) have been shown to regulate nodulation development of the legume hosts by their respective host-specific *Rhizobium* strains (Barker et al. 2002). Thus, a concept has emerged that the genetic pathway of AM symbiosis, in part, is shared by other similar plant-microbe interactions including *Rhizobium* symbiosis (Martin 2001). Around 224 genes are affected during AM symbiosis in rice, and 34% of these genes were also found to be associated with mycorrhization in dicots, revealing a conserved pattern of response (Güimil et al. 2005). The likelihood of some of these AM-responsive genes being evolutionary conserved, since the early days of land plant evolution (Simon et al. 1993), has functional importance in plant biology, indicating the possibility of their exploitation in breeding for symbiosis response.

Another area relevant for exploitation of symbiosis is host genes for enhancing plant response related to phosphate (Pi) transporters. Pi transporters of AMF and plant hosts including rice have been cloned and characterized (Versaw et al. 2002). The researches have shown that some of these plant Pi transporter genes are specifically activated in AM symbiosis, for example, the rice phosphate transporter gene OsPT11 was specifically induced during AM infection. This induction is correlated with the degree of AM colonization and is specifically confined to the root system (Paszowski et al. 2002). Similar evidences have been recorded in potato and tomato plants (Nagy et al. 2005). Among two phosphate transporter genes identified to be involved in AM symbiosis, viz. OsPT11 and OsPT13, the former may be responsible for both AM development and symbiotic phosphate uptake, whereas OsPT13 may act as a sensor to detect the phosphate level appropriate for arbuscule development (Yang et al. 2012). A possibility has thus emerged of exploiting these host-specific or non-specific AM-regulated phosphate

transporter genes for genetic enhancement of nutritional physiology. Rice, in view of its sequenced genome (International Rice Genome Sequencing Project (IRGSP) 2005), seems to be a suitable plant for such model work.

Inter- and intraspecies variation in AMF response (for growth promotion and P acquisition) in cultivars of onion (*Allium fistulosum*) (Tawaraya et al. 2001), other crops (Smith and Read 1997; Koide 1991; McGonigle and Fitter 1990) including cereals like wheat (*Triticum aestivum*) (Hetrick et al. 1995), maize (*Zea mays*), barley (*Hordeum vulgare*) (Gianinazzi-Pearson 1984; Smith and Read 1997) and rice (*Oryza sativa*) (Dhillion 1992; Maiti et al. 1995; Toppo et al. 2015—unpublished) have been observed. Such varietal differences in response suggest that mycorrhizal responsiveness is a genetic character. Sustained research on the genetics of mycorrhiza formation over the last few years has revealed that plant response to mycorrhiza may depend on the genomic backgrounds of the fungus, the plant and their interaction with environment (Franken and Requena 2001). Formal genetic studies with large number of cultivars of *T. aestivum* indicated that ‘mycorrhizal responsiveness’ genes might exist in different chromosomes of some cultivars (Hetrick et al. 1995). In double haploid mapping populations of barley varieties, differences in mycorrhiza responsiveness have been identified by formal genetic approaches (Langbridge et al. 1995). Using ‘near-isogenic lines’ (NILs), similar host genotypic variations in colonization and host response have been identified in white clover (Eason et al. 2001). Inter-cultivar variation in P acquisition due to AMF colonization has been reported in double haploid genetic population of *Hordeum vulgare*, and the presence of ‘quantitative trait loci’ (QTLs) for mycorrhiza responsiveness was indicated (Barker et al. 2002). Subsequently, QTLs for AM responsiveness were identified in crops like *Allium* species (Galvàn et al. 2011) and maize (*Zea mays*) (Kaeppler et al. 2000). Galvàn et al. (2011) identified four genomic regions involved in mycorrhizal responsiveness in onion to *Glomus intraradices*. These QTLs also controlled the plant average performance positively and the number of roots. Response to mycorrhizal fungi in a ‘recombinant inbred line’ (RIL) population of B73 × Mo17 maize population led to identification of three QTLs to influence growth at low P in the absence of mycorrhiza based on shoot weight. These findings have opened up the possibility to utilize this genetic variability to select/breed high AM-responsive crop varieties to exploit the biological potentials of AMF in managing P nutrition. The knowledge of genetic basis of mycorrhiza response would allow genetic breeding/manipulation of the varieties for increased mycorrhizal response in terms of plant supply of phosphorus in an otherwise phosphorus-deficient soil, simply by getting the plants infected with appropriate mycorrhiza, for the maximum P use efficiency of applied chemical P fertilizers.

Analysis of the completed rice genome sequence resulted in identification of literally tens of thousands of new targets for DNA markers, especially SSRs. In rice (*Oryza sativa*), earlier studies reviewed by McCouch et al. (1997) demonstrated that microsatellite markers are distributed relatively uniformly throughout the genome and detect the level of allelic diversity in cultivated varieties and distantly related species. For mapping, genetic analysis and marker-assisted plant improvement

strategies, a total of 2414 new di-, tri- and tetra-nucleotide non-redundant SSR primer pairs representing 2240 unique marker loci have been developed and experimentally validated for rice which is publicly available (McCouch et al. 2002). This was soon followed by 18,828 class I (di-, tri-, tetra-repeats) SSRs that were released after the completion of the Nipponbare genome sequence in 2005 (Matsumoto et al. 2005). This number is by far the largest number of publicly available SSRs for any crop species. The extremely high density of SSRs (approximately 51 SSRs per Mb) provides a considerable 'tool kit' for map construction and marker assisted selection (MAS) of crop variety for numerous applications (Collard and Mackill 2008). SSR molecular markers are frequently used to assess genetic variation within and between populations (Vigouroux et al. 2005), and there have been many studies describing genetic diversity in a wide range of species (Jamil et al. 2013; Prabhakaran et al. 2010; Herrera et al. 2008; El-Malky et al. 2007; Garris et al. 2005). SSRs have also been used in rice DNA fingerprinting (Rahman et al. 2009; Chakravarthi and Naravaneni 2006). There are lot of evidences where SSRs have already been used to identify several qualitative or quantitative trait loci in rice for successful breeding and selections. Zhang et al. (2001) used 315 DNA markers in a population of 154 double haploid lines of rice and could identify 41 QTLs for drought tolerance. Sharma et al. (2005) using such 178 SSR markers to a F_2 genotype population of 208 individuals could map Pi-k (h) gene of rice, which confers resistance to *Magnaporthe oryzae*, the blast disease-causing pathogen. All these evidences are available in the public domain (Gramene literature). Jing et al. (2010) studied QTLs associated with yield-related traits using an 'advanced backcross' population derived from common wild rice (*Oryza rufipogon* L). These markers can be useful in molecular mapping and marker-assisted selection as suggested by Aliyu et al. (2011). In the last few years, a lot of works have been done with these markers for QTL analysis in rice for various traits (Gao and Sun 2013; Sandhu et al. 2013; Zhao et al. 2013; Mararhi et al. 2012; Kebriyae et al. 2012; Rathi et al. 2011; Wan et al. 2008). However, no such works are still noticed for the identification of the trait of AM responsiveness in terms of enhanced phosphorus nutrition and growth promotion particularly for upland rice. For traits as difficult to evaluate as AM responsiveness, molecular markers allow breeders to track the genetic loci linked to such complex traits and help in their indirect selection. Simple sequence repeats (SSRs) for such analysis are considered the molecular markers of choice, due to high level of polymorphism, high reproducibility, codominance, relative abundance and rapid but simple genotyping assays (Kong et al. 2000). SSRs occur as frequently as once in approximately every 6 kb in plant genomes (Cardle et al. 2000) and are highly preferred due their abundant distribution in the genomes examined till date and their hyper-variable nature. Attempts are being made to identify phenotypic plant traits in upland rice varieties most likely associated with AMF-mediated physiological response, which in turn would aid in identification of polymorphic SSR markers between the most significantly different (AM responsiveness) phenotypes (through parental polymorphism), for future usage in QTL analysis for the AM

responsiveness trait in terms of P uptake and growth promotion in the selected upland rice varieties.

6.4 Conclusion and Perspective

Poor phosphorus nutrition of rice, grown particularly in upland ecology, is one major constraint for improving productivity. Phosphorus promotes tillering, root development, early flowering and ripening. Without adequate supply of P, plants cannot reach its maximum yield because it reduces panicles/plant, grains/panicle and filled grain number/panicle. Proper P supply also reduces spikelet sterility (Aide and Picker 1996) resulting in further yield increase. Thus, addition of P fertilizers in large amounts for enhancing yield is commonly practiced all around the world (Itao et al. 1999). But the main problem with P fertilizers is its less mobility and fixation with soil complex within a short period of application rendering more than two-thirds unavailable (Sahrawat et al. 2001). This necessitates access of fixed P to plants for promoting P economy and reducing production cost. Biological way (exploiting arbuscular mycorrhiza) of capturing this untapped P in soil thus has gained importance among researchers as an environment-friendly and climate-resilient (Maiti et al. 2013) avenue. The present document reviewed the prospective ways of capturing ecosystem services rendered by native AM fungus for improving P nutrition of upland rice. The future perspectives of exploiting this biological opportunity by means of trapping host plant genetic quantitative trait of high AM responsiveness for P acquisition has also been discussed which (AM-responsive variety) in combination with AM-supportive crop culture components would promote capturing this ecosystem service to the full extent.

References

- Abdelmoneim TS, Tarek AMM, Almaghrabi OA, Hassan SA, Ismail A (2014) Increasing Plant tolerance to drought stress by inoculation with arbuscular mycorrhizal fungi. *Life Sci J* 10:3273–3280
- Aide M, Picker J (1996) Potassium and phosphorus nutrition in rice. Information from 1996 Missouri Rice Research Update
- Aliyu R, Adamu AK, Muazu S, Alonge SO, Gregorio GB (2011) Tagging and validation of SSR markers to salinity tolerance QTLs in rice (*Oryza* spp.). In: Proceedings of international conference on Biology, Environment and Chemistry, vol 1. IACSIT Press, Singapore
- Ambriz E, Baez-Perez A, Sanchez-Yanez JM, Moutoglis P, Villegas J (2010) *Fraxinus-Glomus-Pisolithus* symbiosis: plant growth and soil aggregation effects. *Pedobiologia* 53:369–373
- Auge RM, Duan X, Ebel RC, AJW S (1994) Nonhydraulic signalling of soil drying in mycorrhizal maize. *Planta* 193:74–82
- Bagyaraj DJ, Sharma MP, Maiti D (2015) Phosphorus nutrition of crops through arbuscular mycorrhizal fungi. *Curr Sci* 109:1288–1293

- Balzerque C, Puech-Pagès V, Bécard G, Rochange SF (2011) The regulation of arbuscular mycorrhizal symbiosis by phosphate in pea involves early and systemic signalling events. *J Exp Bot* 62:1049–1060
- Baqual MF, Das PK (2006) Influence of Biofertilizers on macronutrient uptake by the mulberry plant and its impact on silkworm bioassay. *Caspian J Environ Sci* 4:98–109
- Barker SJ, Duplessis S, Tagu D (2002) The application of genetic approaches for investigations of mycorrhizal symbiosis. *Plant Soil* 244:85–95
- Burleigh SH, Bechmann IE (2002) Plant nutrient transporter regulation in arbuscular mycorrhizas. *Plant Soil* 244:247–251
- Cardle L, Ramsay L, Milbourne D, Macaulay M, Marshall D, Waugh R (2000) Computational and experimental characterization of physically clustered simple sequence repeats in plants. *Genetics* 156:847–854
- Cavagnaro TR, Franz Bender S, Asghari HR, Van der Heijden MGA (2015) The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends Plant Sci* 20:283–290. doi:[10.1016/j.tplants.2015.03.004](https://doi.org/10.1016/j.tplants.2015.03.004)
- Chakravarthi BK, Naravaneni R (2006) SSR marker based DNA fingerprinting and diversity study in rice (*Oryzasativa* L). *Afr J Biotechnol* 5:684–688
- Collard BCY, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil Trans R Soc B* 363:557–572
- Davies FT, Olalde-Portugal V, Aguilera-Gomez L, Alvarado MJ, Ferrera-Cerrato RC, Boutton TW (2002) Alleviation of drought stress of Chile ancho pepper (*Capsicum annum* L. cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico. *Sci Hortic* 92:347–359
- Degens BP, Sparling GP, Abbott LK (1996) Increasing the length of hyphae in a sandy soil increases the amount of water-stable aggregates. *Appl Soil Ecol* 3:149–159
- Dhillion SS (1992) Host endophyte specificity of vesicular arbuscular mycorrhizal colonization of *Oryzasativa* L. at the pre-transplant stage in low or high phosphorus soil. *Soil Biol Biochem* 24:405–411
- Diaz G, Azcón-Aguilar C, Honnubia M (1996) Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. *Plant Soil* 180:241–249
- Douds DD Jr, Nagahashi G, Pfeffer PE, Kayser WM, Reider C (2005) On-farm production and utilization of arbuscular mycorrhizal fungus inoculum. *Can J Plant Sci* 85:15–21
- Douds DD Jr, Nagahashi G, Pfeffer PE, Reider C, Kayser WM (2006) On-farm production of AM fungus inoculum in mixtures of compost and vermiculite. *Bioresour Technol* 97:809–818
- Eason WR, Webb KJ, Michaelson-Yeates TPT, Abberton MT, Griffith GW, Culshaw CM, Hooker JE, Dhanoa MS (2001) Effect of genotype of *Trifolium repens* on mycorrhizal symbiosis with *Glomus mosse*. *J Agric Sci (Camb)* 137:27–36
- El-Malky MM, Fahmi AI, Kotb AA (2007) Detection of genetic diversity using microsatellites in rice (*Oryza sativa* L.) African Crop Science Conference Proceedings 8:597–603
- Fageria NK, Barbosa Filho MP, Catvalho JRP (1982) Response of upland rice to phosphorus fertilization on an Oxisol of Central Brazil. *Agron J* 74:51–56
- Fattah OA (2013) Effect of Mycorrhiza and phosphorus on micronutrients uptake by soyabean plant grown in acid soil. *Int J Agron Plant Prod* 4:429–437
- Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z (2002) Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* 12:185–190
- Fontenla S, Puntieri J, Ocampo JA (2001) Mycorrhizal associations in the Patagonian steppe, Argentina. *Plant Soil* 233:13–29
- Franken P, Requena N (2001) Analysis of gene expression in arbuscular mycorrhizas: new approaches and challenges. *New Phytol* 150:517–523
- Galván GA, Kuyper TW, Burger K, Paul Keizer LC, Hoekstra RF, Kik C, Scholten OE (2011) Genetic analysis of the interaction between *Allium* species and arbuscular mycorrhizal fungi. *Theor Appl Genet* 122:947–960. doi:[10.1007/s00122-010-1501-8](https://doi.org/10.1007/s00122-010-1501-8)

- Gao D, Sun L (2013) In vitro screening and molecular characterization of a bacterial blight resistance gene in rice. *J Rice Res* 1:104. doi:[10.4172/jrr.1000104](https://doi.org/10.4172/jrr.1000104)
- Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S (2005) Genetic structure and diversity in *Oryzasativa* L. *Genetics* 169:1631–1638
- Gaur A (1997) Inoculum production technology development of vesicular-arbuscular mycorrhizae. PhD Thesis, Department of Botany, University of Delhi, Delhi
- Gaur A, Adholeya A (2002) Arbuscular mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biol Fertil Soils* 35:214–218. doi:[10.1007/s00374-002-0457-5](https://doi.org/10.1007/s00374-002-0457-5)
- Gaur A, Adholeya A, Mukerji KG (2000) On-farm production of VAM inoculum and vegetable crops in marginal soil amended with organic matter. *Trop Agric* 77:21–26
- Gianinazzi S, Gollotte A, Binet MN, Tuinen D, van Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* 20:519–530
- Gianinazzi-Pearson V (1984) Host-fungus specificity, recognition and compatibility in mycorrhizae. In: Verma DPS, Hohn T (eds) *Genes involved in microbe–plant interactions*. Springer, Wien, pp 225–253
- Goltapeh EM, Danesh YR, Prasad R, Varma A (2008) Mycorrhizal fungi: what we know and what should we know? In: Varma A (ed) *Mycorrhiza*. Springer, Berlin, pp 3–27. doi:[10.1007/978-3-540-78826-3_1](https://doi.org/10.1007/978-3-540-78826-3_1)
- Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. *Agric Ecosyst Environ* 113:17–35
- Graham JH, Leonard RT, Menge JA (1981) Membrane mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiol* 68:548–552
- Güimil S, Chang H, Zhu T, Sesma A, Osbourn A, Roux C, Ioannidis V, Oakely EJ, Docquier M, Descombes P, Briggs SP, Paszkowski U (2005) Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. *Proc Natl Acad Sci USA* 102:8066–8070
- Gupta PC, O’Toole JC (1986) Upland rice distribution. In: *Upland rice: a global perspective*, International Rice Research Institute Publications, Los Baños, Phillipines, pp 1–11
- Habte M, Manjunath A (1987) Soil solution phosphorus status and mycorrhizal dependency in *Leucaenaleuco cephal*a. *Appl Environ Microbiol* 53:797–801
- Harinikumar KM, Bagyaraj DJ (2005) Effect of crop rotation on native arbuscular mycorrhizal propagules in soil. *Plant Soil* 110:77–80
- Harrier LA, Watson CA (2003) The role of arbscular mycorrhizal fungi in sustainable cropping systems. *Adv Agron* 79:185–225
- Herrera TG, Duque DP, Almeida IP, Nunez GT, Pieters AJ, Martinez CP, Tohme JM (2008) Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeat markers. *Electron J Biotechnol* 11 special issue
- Hetrick BAD, Wilson GWT, Gill BS, Cox TS (1995) Chromosome location of mycorrhizal responsive genes in wheat. *Can J Bot* 73:891–897
- Hildebrandt U, Marjana R, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry* 68:139–146
- Huang J, Lou S, Zeng R (2003) Mechanism of plant disease resistance induced by arbuscular mycorrhizal fungi. *Ying Yong Sheng Tai XueBao* 14:819–822
- International Rice Genome Sequencing Project (IRGSP) (2005) <http://pgir.rutgers.edu/IRGSP.html>
- International Rice Research Institute (1975) Major research in upland rice. International Rice Research Institute Publications, Los Baños, Philippines, 255 p
- Itao E, Ella E, Kawanto N (1999) Physiological basis of submergence tolerance in rainfed lowland rice ecosystem. *Field Crop Res* 64:75–90

- Izaguirre-Mayoral ML, Carballo O, Carreno L, de Mejia MG (2000) Effects of arbuscular mycorrhizal inoculation on growth, yield, nitrogen, and phosphorus nutrition of nodulating bean varieties in two soil substrates of contrasting fertility. *J Plant Nutr* 23:1117–1133
- Jamil M, Rana IA, Ali Z, Awan FS, Shahzad Z, Khan AS (2013) Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using Simple Sequence Repeats. *Mol Plant Breed* 4:285–291
- Janos DP (1988) Mycorrhiza applications in tropical forestry: are temperate-zone approaches appropriate? In: FSP N (ed) *Trees and mycorrhiza*. Forest Research Institute, Kullalumpur, pp 133–188
- Jasper DA, Abbot LK, Robson AD (1991) The effect of soil disturbance on vesicular arbuscular mycorrhizal fungi in soils from different vegetation type. *New Phytol* 118:471–476
- Jing Z, Qu Y, Yu C, Pan D, Fan Z, Chen Z, Li C (2010) QTL analysis of yield-associated traits using an advanced backcross population derived from common wild rice (*Oryza rufipogon* L.). *Mol Plant Breed* 1(1). doi:10.5376/mpb.2010.01.0001
- Johansen A, Jakobsen I, Jensen ES (1993) External hyphae of vesicular arbuscular mycorrhizal fungi associated with *Trifolium subterranean* L. and hyphal transport of ^{32}P and ^{15}N . *New Phytol* 7:365–386
- Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol Ecol* 48:1–13
- Kaeppeler SM, Parke JL, Mueller SM, Sr L, Charles S, Tracy F (2000) Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. *Crop Sci* 40:358–364
- Kahiluoto H, Vestberg M (1998) The effect of arbuscular mycorrhiza on biomass production and phosphorus uptake from sparingly soluble sources by leek (*Allium porrum* L.) in Finnish field soil. *Biol Agric Hortic* 16:65–85
- Kahiluoto H, Vestberg M (1999) Methods to create a non-mycorrhizal control for a bioassay of AM effectiveness. 2. Benomyl application and soil sampling time. *Mycorrhiza* 9:259–270
- Kahiluoto H, Ketoja E, Vestberg M (2000) Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization 1. Bioassays in a growth chamber. *Plant Soil* 227:191–206
- Kahiluoto H, Ketoja E, Vestberg M (2001) Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant Soil* 231:65–79
- Kebriyae D, Kordrostami M, Rezadoost MH, Lahiji HS (2012) QTL analysis of agronomic traits in rice using SSR and AFLP markers. *Notulae Scientia Biologicae* 4:116–123
- Khaliiq A, Sanders FE (2000) Effects of vesicular-arbuscular mycorrhizal inoculation on the yield and phosphorus uptake of field-grown barley. *Soil Biol Biochem* 32:1691–1696
- Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84:2292–2301
- Koide R (1991) Nutrient supply, nutrient demand and plant-response to mycorrhizal infection. *New Phytol* 117:365–386
- Kong L, Dong J, Hart GE (2000) Characteristics, linkage-map positions and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple sequence repeats (SSRs). *Theor Appl Genet* 101:438–448
- Kruckelmann W (1975) Effects of fertilizers, soils, soil tillage and plant species on the frequency of Endogone chlamydospores and mycorrhizal infection in arable soils. In: Sanders FE, Mosse B, Tinker PB (eds) *Endo-mycorrhizas*. Academic Press, London, pp 511–535
- Langbridge P, Karakousis A, Collins N, Kretschmer J, Manning S (1995) A consensus linkage map of barley. *Mol Breed* 1:389–395
- Li XL, George E, Marschner H (1991a) Extension of the phosphorus depletion zone in VA-mycorrhizal clover in calcareous soil. *Plant Soil* 136:41–48
- Li XL, Marschner H, George E (1991b) Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover. *Plant Soil* 136:49–57
- Lin AJ, Zhang XH, Wong MH, Ye ZH, Lou LQ, Wang YS, Zhu YG (2007) Increase of multi-metal tolerance of three leguminous plants by arbuscular mycorrhizal fungi colonization. *Environ Geochem Health* 29:473–481

- Lingua G, D'Agostino G, Massa N, Antosiano M, Berta G (2002) Mycorrhiza-induced differential response to a yellows disease in tomato. *Mycorrhiza* 12:191–198
- Maiti D (2011) Improving activity of native arbuscular mycorrhizal fungi (AMF) for mycorrhizal benefits in agriculture: status and prospect. *J Biofertil Biopestici (S1-001)* 2:113. doi:[10.4172/2155-6202.S1-001](https://doi.org/10.4172/2155-6202.S1-001)
- Maiti D, Barnwal MK (2012) Optimization of phosphorus level for effective arbuscular-mycorrhizal activity in rainfed upland rice based cropping system. *Ind Phytopath* 65:334–339
- Maiti D, Variar M, Saha J (1995) Colonization of upland rice by native VAM under rainfed mono-cropped ecosystem. In: Roy AK, Sinha KK (eds) Recent advances in phytopathological research, M.D. Publications, New Delhi, pp 45–52
- Maiti D, Variar M, Singh RK (1996) Perpetuation of native VAM fungi under mono-cropped, rainfed upland agro-ecosystem. *Mycorrhiza News* 8:7–9
- Maiti D, Barnwal MK, Rana SK, Variar M, Singh RK (2006) Enhancing native arbuscular mycorrhizal association to improve phosphorus nutrition of rainfed upland rice (*Oryza sativa* L.) through cropping systems. *Ind Phytopath* 59:432–438
- Maiti D, Barnwal MK, Singh RK (2008) Exploring possibility of utilizing native arbuscular mycorrhizal fungi for improving phosphorus nutrition in transplanted rice (*Oryzasativa* L.) of plateau region. *Ind Phytopath* 61:302–304
- Maiti D, Barnwal MK, Mandal NP (2009a) Exploring possibilities of partial drought mitigation in upland rice (*Oryza sativa* L.) through enhancing native arbuscular mycorrhizal (AM) association. *Mycorrhiza News* 21:29–30
- Maiti D, Barnwal MK, Singh RK, Variar M (2009b) A new protocol for on-farm production method of arbuscular mycorrhizal fungal mass inoculum for rainfed upland rice. *Ind Phytopath* 62:31–36
- Maiti D, Toppo NN, Variar M (2011a) Integration of crop rotation and arbuscular mycorrhizal (AM) fungal inoculum application for enhancing native AM activity to improve phosphorus nutrition of upland rice (*Oryzasativa* L.) *Mycorrhiza* 21:659–667
- Maiti D, Variar M, Singh RK (2011b) Optimizing tillage schedule for maintaining activity of the arbuscular-mycorrhizal fungal population in a rainfed upland rice (*Oryzasativa* L.) agro-ecosystem. *Mycorrhiza* 21(3):167–171. doi:[10.1007/s00572-010-0324-4](https://doi.org/10.1007/s00572-010-0324-4)
- Maiti D, Variar M, Singh RK (2012) Rice based crop rotation for enhancing native arbuscular mycorrhizal (AM) activity to improve phosphorus nutrition of upland rice (*Oryza sativa* L.) *Biol Fert Soils* 48:67–73. doi:[10.1007/s00374-011-0609-6](https://doi.org/10.1007/s00374-011-0609-6)
- Maiti D, Singh CV, Variar M, Mandal NP, Anantha MS (2013) Impact of rainfall pattern on native arbuscular-mycorrhizal activity influencing phosphorus utilization by direct seeded rainfed upland rice. *Proc Natl Acad Sci India Sect B Biol Sci* 83:159–162
- Mararathi B, Guleria S, Mohapatra T, Parsad R, Mariappan N, Kurungara VK, Atwal SS, Prabhu KV, Singh NK (2012) QTL analysis of novel genomic regions associated with yield and yield related traits in new plant type based recombinant inbred lines of rice (*Oryza sativa* L.) *BMC Plant Biol* 12:137. doi:[10.1186/1471-2229-12-137](https://doi.org/10.1186/1471-2229-12-137)
- Marleen I, Sylvie C, Stephane D (2011) Methods for large scale production of AM fungi: past, present and future. *Mycorrhiza* 21:1–16
- Marschner H (1995) Mineral nutrition of higher plants. Academic Press, Cambridge. ISBN: 978-0-12-473542-2
- Marsh JF, Schultze M (2001) Analysis of arbuscular mycorrhizas using symbiosis-defective plant mutants. *New Phytol* 150:525–532
- Martin F (2001) Frontiers in molecular mycorrhizal research genes, loci, dots and spins. *New Phytol* 150:499–507
- Matsumoto T, Wu J, Kanamori H (2005) The map-based sequence of the rice genome. *Nature* 436:793–800

- McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho Y, Huang N, Ishii T, Blair M (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Mol Biol* 35:89–99
- McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Watton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, Declereck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.) *DNA Res* 9:199–207
- McGonigle TP, Fitter AH (1990) Ecological specificity of vesicular-arbuscular mycorrhizal associations. *Mycol Res* 94:120–122
- McGonigle TP, Millar MH (1993a) Mycorrhizal development and phosphorus absorption in maize under conventional and reduced tillage. *Soil Sci Soc Am J* 57:1002–1006
- McGonigle TP, Millar MH (1993b) Response of mycorrhizae and shoot phosphorus of maize to the frequency and timing of soil disturbance. *Mycorrhiza* 4:63–68
- Menendez AB, Scervino JM, Godeas AM (2001) Arbuscular mycorrhiza populations associated with natural and cultivated vegetation on a site of Buenos Aires province. *Argentina Biol Fertil Soil* 33:373–381
- Miller RM, Jastrow JD (1992) The application of VA mycorrhizae to ecosystem restoration and reclamation. In: Allen MF (ed) *Mycorrhizal functioning*. Chapman & Hall., London, pp 438–467
- Mohammad MJ, Malkawi HI, Shibli R (2003) Effects of mycorrhizal fungi and phosphorus fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. *J Plant Nutr* 26:125–137
- Nagy R, Karandashov V, Chague V, Kalinkevich K, Tamasloukht M, Guohua X, Jakobsen I, Avraham AL, Amrhein N, Bucher M (2005) The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. *Plant J* 42:236–250
- Nagy R, Drissner D, Amrhein N, Jakobsen I, Bucher M (2009) Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. *New Phytol* 181:950–959
- Ocampo JA, Hayman DS (1981) Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. *New Phytol* 87:333–343
- Ocampo A, Martin J, Hayman D (1980) Influence of plant interaction on vesicular-arbuscular mycorrhizal infections. I. Host and non-host plants grown together. *New Phytol* 84:27–35
- Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A (2005) Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytol* 165:273–283
- Oliveira RS, Vosatka M, Dodd JC, Castro PMC (2005) Studies on the diversity of arbuscular mycorrhizal fungi and the efficacy of two native isolates in a highly alkaline anthropogenic sediment. *Mycorrhiza* 16:23–31
- Paszowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *PNAS (USA)* 19:13324–13329
- Prabhakaran A, Paramasivam K, Rajesh T, Rajarajan D (2010) Molecular characterization of rice land races using SSR markers. *Electron J Plant Breed* 1:512–516
- Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) *Plant growth-promoting rhizobacteria and medicinal plants*. Springer, Cham, pp 247–260
- Prasad R, Bholra D, Akdi K, Cruz C, Sairam KVSS, Tuteja N and Varma A (2017) Introduction to mycorrhiza: Historical development. In: *Mycorrhiza* (eds. Varma A, Prasad R and Tuteja N) Springer International Publishing Switzerland 1-7
- Rahman MS, Molla MR, Alam MS, Rahman L (2009) DNA fingerprinting of rice (*Oryzasativa* L.) cultivars using microsatellite markers. *Aust J Crop Sci* 3:122–128

- Rana SK, Maiti D, Barnwal MK, Singh RK, Variar M (2002) Effect of rice (*Oryzasativa* L.)-based cropping systems on vesicular arbuscular mycorrhizal colonization, P uptake and yield. *Ind J Agric Sci* 72:400–403
- Rangarajan M, Santhanakrishnan P (1995) Plant growth promoting Rhizobacteria and biofertilizers increase the fresh leaf yield and nutrient content in *Morusalba*. In: Adholeys A, Singh S (eds) Proceedings of the third national conference on mycorrhiza. TERI, New Delhi, pp 189–191
- Rathi S, Baruah AR, Chowdhury RK, Sarma RN (2011) QTL analysis of seed dormancy in indigenous rice of Assam. *India Cereal Res Commun* 39:137–146
- Ravenskov S, Jakobsen I (1995) Functional compatibility in arbuscular mycorrhizas as hyphal P transport to plant. *New Phytol* 129:611–618
- Requena N, Perez-Solis E, Azcon-Aguilar C, Jeffries P, Barea JM (2001) Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Appl Environ Microbiol* 67:495–498
- Richardson AE, Lynch JP, Ryan PR, Delhaize E, Smith FA (2011) Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil* 349:121–156
- Sahrawat KL, Abekoe MK, Diatta S, Tian G, Ishida F, Keatinge D, Carsky R, Wendt J (2001) Application of inorganic phosphorus fertilizer. In: Proceedings of the Symposium Sponsored by the American Society, Argon, USA, 5–9 Nov 2009, pp 225–246
- Salim ME, Mohamed HA, Gamal MA, Siddiqui MH (2013) Role of mycorrhizal fungi in tolerance of wheat genotypes to salt stress. *Afr J Microbiol Res* 7:1286–1295
- Sally ES, Jakobsen I, Grönlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol* 156:1050–1057
- Sandhu N, Jain S, Kumar A, Mehla BS, Jain R (2013) Genetic variation, linkage mapping of QTL and correlation studies for yield, root and agronomic traits for adaptation. *BMC Genet* 14:104. doi:10.1186/1471-2156-14-104
- Schwartz MW, Horksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbot LK, Pringle A (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecol Lett* 9:501–515
- Sharma TR, Madhav MS, Singh BK, Shankar P, Jana TK, Dalal V, Pandit A, Singh A, Gaikwad K, Upreti HC, Singh NK (2005) High-resolution mapping, cloning and molecular characterization of the Pi-k (h) gene of rice, which confers resistance to *Magnaporthe grisea*. *Mol Gen Genomics* 274:569–578
- Sharma SK, Ramesh A, Sharma MP, Joshi OP, Govaerts B, Steenwearth KL, Karlen DL (2010) Microbial community structure and diversity as indicators for evaluating soil quality. In: Lichtfouse E (ed) Biodiversity, biofuel, agroforestry and conservation agriculture: sustainable agriculture reviews. Springer, Dordrecht, pp 317–358. doi:10.1007/978-90-481-9513-8_11
- Shukla A, Kumar A, Jha A, Nageawar Rao DVK (2011) Phosphorus threshold for arbuscular-mycorrhizal colonization of crops and tree seedlings. *Biol Fertil Soils* 48:109–116
- Siddiqui ZA, Pichtel J (2008) Mycorrhizae: an overview. *Sustainable agriculture and forestry*. Springer, Dordrecht, pp 1–35
- Sieverdin E (1991) Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn, 371 p
- Simon L, Bousquet J, Levesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with land plants. *Nature* 363:67–69
- Siqueira JO, Saggin OJ Jr (2001) Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. *Mycorrhiza* 11:245–255
- Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, London, 605 p. ISBN: 0-12-652840-3
- Smith FA, Smith SE (2011) What is the significance of the arbuscular mycorrhizal colonization of many economically important crop plants? *Plant Soil* 348:63–79

- Srimathi PL, Kumutha K, Arthee R, Pandiyarajan P (2014) Studies on the role of arbuscular mycorrhiza fungal enhancement on soil aggregate stability. *Res J Recent Sci* 3:19–28
- Tawarayama K, Tokairin K, Wagatsuma T (2001) Dependence of *Allium fistulosum* cultivars on the arbuscular mycorrhizal fungus *Glomus fasciculatum*. *Appl Soil Ecol* 17:119–124
- Thompson JP (1987) Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. *Aust J Agric Res* 38:847–867
- Tisdall JM (1991) Fungal hyphae and structural stability of soil. *Aust J Soil Res* 29:729–743
- Toppo NN, Maiti D (2011) Native arbuscular mycorrhizal fungal diversity in rice based cropping systems under rainfed ecology (Abstr.). In: Proceedings of the International Conference on “Microbial Biotech. for sustainable development”, PU, Chandigarh, India, 3–6 Nov 2011, p 415
- Toppo NN, Srivastava AK, Maiti D (2013) Effect of arbuscular mycorrhizal (AM) inoculation on upland rice root system. *The Bioscan* 8:533–536
- Umakant GC, Bagyaraj DJ (1998) Response of mulberry saplings to inoculation with VA mycorrhizal fungi and Azotobacter. *Sericologia* 38:669–675
- Verbruggen E, Kiers ET (2010) Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evol Appl* 3:547–560
- Versaw WK, Chiou TJ, Harrison MJ (2002) Phosphate transporters of *Medicago truncatula* and arbuscular mycorrhizal fungi. *Plant Soil* 244:239–245
- Vierheilig H (2004) Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. *Can J Bot* 82:1166–1176
- Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S, Smith JSC, Jaqueth J, Smith OS, Doebley J (2005) An analysis of genetic diversity across the maize genome using microsatellites. *Genetics* 169:1617–1630
- Vosatka M, Rydlova J, Sudova R, Vohnik M (2006) Mycorrhizal fungi as helping agents in phytoremediation of degraded and contaminated soils. In: Mackova M, Dowling DN, Mace T (eds) *Phytoremediation and rhizoremediation*. Springer, Berlin, pp 237–255
- Wan X, Weng J, Zhai H, Wang J, Lei C, Liu X, Guo T, Jiang L, Su N, Wan J (2008) Quantitative trait loci (QTL) analysis for rice grain width and fine mapping of an identified QTL allele gw-5 in a recombination hotspot region on chromosome 5. *Genetics* 179:2239–2252
- Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Can J Bot* 82:1198–1227
- Wilcox HE (1991) Mycorrhizae. In: Waisel Y, Eshel A, Kafkati U (eds) *The plant root: the hidden half*. Marcel Dekker, New York, pp 731–765
- Wright SF, Upadhyaya AA (1996) Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci* 16:575–586
- Wu TH, Hao WY, Lin XG, Shi YQ (2002) Screening of arbuscular mycorrhizal fungi for the re-vegetation of eroded red soils in subtropical China. *Plant Soil* 239:225–235
- Yang SY, Grønlund M, Jakobsen I, Grottemeyer MS, Rentsch D, Miyao A, Hirochika H, Kumar CS, Sundaresan V, Salamin N, Catausan S, Mattes N, Heuer S, Paszkowski U (2012) Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the phosphate transporter1 gene family. *Plant Cell* 24:4236–4251
- Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sarkarung S, Blum A, Nguyen HT (2001) Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theor Appl Genet* 103:19–29
- Zhao X, Qin Y, Jia B, Kim SM, Lee HS, Eun MY, Kim KM, Sohn JK (2013) Comparison and analysis of QTLs, epistatic effects and QTL × environment interactions for yield traits using DH and RILs populations in rice. *J Integr Agric* 12:198–208

Chapter 7

Arbuscular Mycorrhizal Fungi in Redeeming Arsenic Toxicity in Plants

Surbhi Sharma, Neeraja Singh, and Rupam Kapoor

Abstract Arsenic (As) contamination has transitioned into a global threat, hampering the survival of millions. Chemical fixation/remediation techniques have proved to be inadequate to reduce As toxicity. Use of arbuscular mycorrhizal fungi (AMF) in alleviation of As stress is a reliable and efficient approach. AMF have been reported to be present in As contaminated soils and are known to exert ameliorative role on detrimental effects of As. Although presence of As in soil affects AMF spore germination and colonization, they have been found to occur even in highly contaminated soils. AMF alleviate As toxicity by extending its extraradical mycelium beyond the depletion zone and help in the uptake of various nutrients increasing the biomass of the plant. AMF sequester As in its various fungal structures such as intraradical hyphae, arbuscules and vesicles preventing their translocation to aerial plant parts. Arsenate [As(V)] and inorganic P (Pi) compete for the same transport proteins in root plasma membrane. AMF could decrease As(V) uptake into the roots by suppressing the high affinity As (V)/(Pi) transporters. It thus enhances the P-uptake by circumventing the direct pathway and channelizing P-uptake by mycorrhizal pathway. AMF results in As stress tolerance in plants by enhancing P uptake, biotransformation of As(V), reduced As uptake, sequestration, protection from oxidative damage and improved physiology of plants.

7.1 Introduction

One of the toxic metalloids distributed widely in the environment is Arsenic (As) (Huysman and Frankenberger 1990; Phillips 1990; Mahimairaja et al. 2005). The main environmental exposure to As for humans is through contaminated drinking water (Meharg et al. 2009), for instance, in the Indian sub-continent As in drinking water has become a huge problem (Nordstrom 2002). Its entry in the environment can occur by natural activities (rock and soil erosion, volcanic action)

S. Sharma • N. Singh • R. Kapoor (✉)
Department of Botany, University of Delhi, New Delhi 110007, India
e-mail: kapoor_rupam@yahoo.com

or industrial and agricultural practices (fertilizers, pesticides, herbicides, mining) (Adriano 2001; Mandal and Suzuki 2002).

Arsenic is generally toxic to plants and is non-essential element. The predominant form of As available for uptake by plants is arsenate [As(V)] and arsenite [As(III)] (Zhao et al. 2009). Once in the cell, As(V) can be readily converted to As(III), the more toxic of the two forms (Gonzalez-Chavez Mdel et al. 2011, 2014). As (V) and As(III) both disrupt plant metabolism, but through different mechanisms. Roots are usually the first tissue to be exposed to As, where the metal inhibits root proliferation and extension. Upon translocation to the shoot, As can severely inhibit plant growth by reducing or arresting biomass accumulation and expansion, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production (Garg and Singla 2011). At higher concentrations, As interferes with vital metabolic and physiological processes, which can ultimately lead to cell death.

In its defense, plant launches antioxidant machinery. The various enzymatic and non-enzymatic antioxidants work in congruence to rescue plant from the oxidative stress that As sets in (Sairam et al. 2005; Sharma et al. 2007; Gunes et al. 2009). Other mechanisms such as complex formation with phytochelatins (PCs) and metallothioneins and the subsequent sequestration in the vacuole and transformation of inorganic As into less toxic organic methyl arsenates also contribute (Gonzalez-Chavez et al. 2002) to plant's tolerance to As toxicity (Fig. 7.1)

Arbuscular mycorrhizal fungi (AMF) have multifarious roles, besides providing mineral nutritional; they bequeath host plant with biotic and abiotic stress tolerance (Fig. 7.2). Several studies have highlighted the role of AMF in advocating As tolerance in plants (Covey et al. 1981; Chen et al. 2007; Xia et al. 2007; Hua et al. 2009). However, there are voids in the understanding of functional and structural contribution of AMF in As stress amelioration, more so because the interaction of AMF and plant is species specific in nature.

The present chapter provides a comprehensive review of menace caused due to As contamination globally, As acquisition, and uptake in plants and its detrimental effects. Particular emphasis is given on the mechanisms employed by AMF for alleviation of As toxicity.

7.2 Arsenic Menace

Arsenic has been identified as the most prevalent contaminant found in soils (Shaibur et al. 2008). It ranks 20th, 14th, and 12th in the earth's crust, sea water, and human body, respectively (Mandal and Suzuki 2002). However, the established average As content of earth's crust is 2.5 mg/kg (NAS 2000); it is found to be present at a concentration of 45 to 3275 mg/kg (Nagy et al. 2005). Increased concentrations of As in water and soil have become a menace at the global level. The most significant occurrence of As is seen in India, Bangladesh, Nepal, Northern

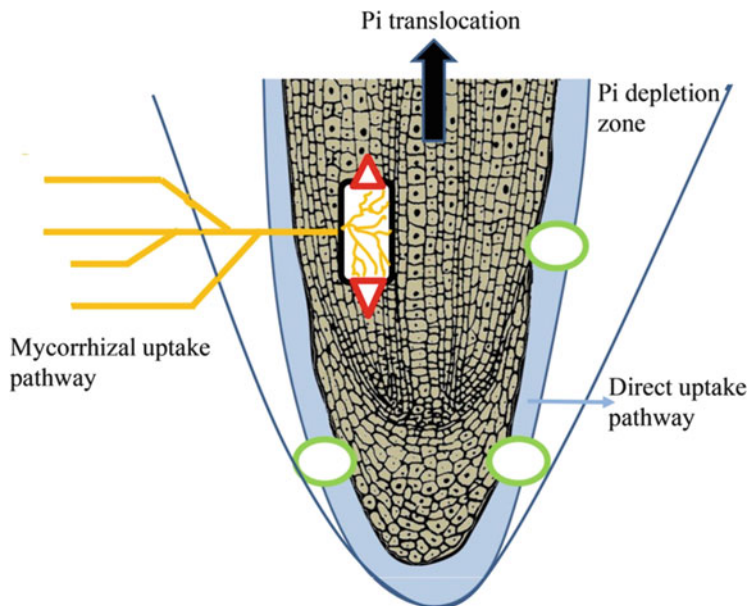


Fig. 7.1 Schematic representation of inorganic orthophosphate (Pi) uptake pathway in an arbuscular mycorrhizal root. Rapid uptake of Pi by direct pathway occurs through Pht1 (green circles) transporters located at epidermis and root hairs cells. Rate of Pi uptake surpasses the rate of diffusion, resulting in the formation of depletion zone. In the mycorrhizal uptake pathway, extraradical fungal hyphae extend beyond the depletion zone. With the help of fungal transporters located in extraradical hyphae, they help in Pi uptake. Pi reaches the symbiotic interface in the root cortex and to intracellularly present arbuscules. At this interface, Pht1 transporters (red triangles) help in Pi absorption in root cortical cells

China, Myanmar, and Vietnam (Das et al. 2004; Patel et al. 2005; Hasanuzzaman and Fujita 2012).

The condition of As toxicity in India is alarming with reports of severe health problems in the populations of West Bengal, Bihar, Jharkhand, and Assam (Acharya 2002; Chakraborti et al. 2008, 2013; Roy et al. 2014; Kumar et al. 2015). Arsenic occurrences in groundwater of Bengal Delta Plain (i.e., Bangladesh and West Bengal) is amongst the largest environmental health disaster encountered recently, where approximately 50 million people are at risk of cancer and other As related diseases due to the consumption of high As contaminated groundwater and food (Singh 2015).

Inorganic As has been categorized by the US Environmental Protection Agency (EPA 1988) and International Agency for Research on Cancer (IARC 1980, 1987) as a class I carcinogen (Hughes 2002). Chronic consumption of As contaminated drinking water leads to cancer of internal organs. Bladder, liver, and kidney are common tumor sites (Smith et al. 1992; Bates et al. 1995). Chronic oral exposure to As causes skin lesions, which are characterized by hyperpigmentation, hyperkeratosis, and hypopigmentation (Yeh et al. 1968; Cebrian et al. 1983).

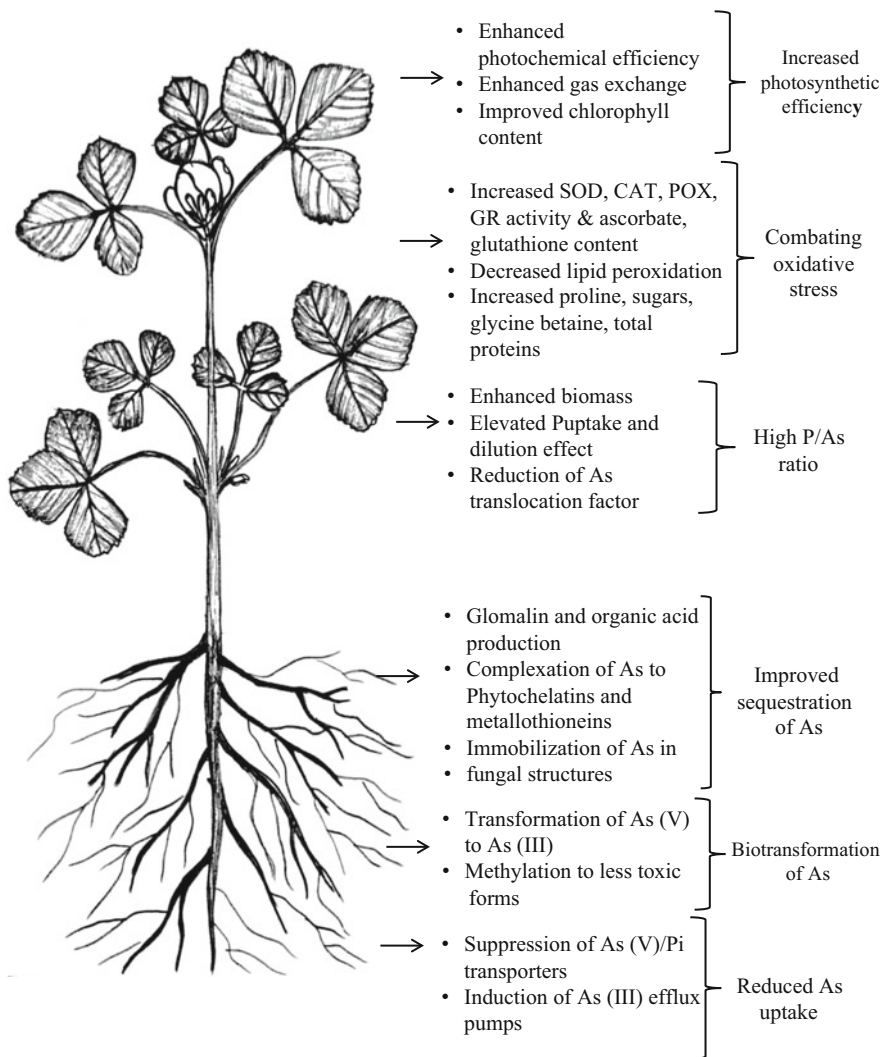


Fig. 7.2 Schematic representation of arbuscular mycorrhiza in alleviation of arsenic toxicity in plants

7.3 Arsenic Uptake in Plants

Arsenic exists in natural systems in both inorganic and organic forms. In inorganic forms, it occurs as trivalent As(III) and pentavalent As(V) state. As (V) predominates in aerobic environments while As(III) predominates in anaerobic environments (Cullen and Reimer 1989). Arsenate can convert to As(III) and *vice versa* based on the redox state and pH of the environment (Zhao et al. 2010).

Certain microorganisms and algae are known to methylate inorganic As(III) present in soil (Bentley and Chasteen 2002). The organic forms of As found in soil are monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO). These organic species generally have low levels (Takamatsu et al. 1982; Huang and Matzner 2006), but can approach higher concentrations too (Abedin et al. 2002). The methylated forms of As(V) can be reduced to more toxic methylated As(III) forms, which pose a great concern as they are commonly found in the environment.

In plants, As accumulation is influenced by its availability in the soil and metabolic as well as physiological properties of the plant (Kumar et al. 2015). Arsenate is an analog of inorganic phosphate (Pi) and, thus, is readily transported across the plasma membrane by phosphate transporters proteins (PHT) (Meharg and Hartley-Whitaker 2002; Smith et al. 2010a; Wu et al. 2011a, b). Under low Pi conditions, As(V) may outcompete Pi for entry into the plant, thus resulting in intensification of Pi deficiency symptoms (Tut and Ma 2003; Catarcha et al. 2007). Arsenate toxicity has been reported to be changed by genetically manipulating the PHT protein concentration (Shin et al. 2004; Gonzalez et al. 2005; Wu et al. 2007).

Arsenite enters root cells through nodulin 26-like intrinsic proteins (NIPS) Meharg and Jardine 2003. These proteins belong to the aquaporin family of major intrinsic proteins (Bienert et al. 2008; Ma et al. 2008). Arsenate shares its transport pathway with Pi while As(III) shares its pathway with silicon (Si). In rice roots, OsNIP2;1/OsLsi 1 Si transporter provides major entry route for As(III) uptake, and OsLsi2 mediates As(III) efflux from exodermis towards stele. Reduction of intracellular As(V) to As(III) occurs by the action of an enzyme arsenate reductase (Bleeker et al. 2006). Arsenite then combines with thiol (-SH) compounds to form As-thiolates, which are then transported and sequestered in the vacuoles (Mukhopadhyay et al. 2000).

Similar to As(III), methylated As species enter roots via the aquaporin channels (Li et al. 2009). Although uptake by roots is much slower for MMA and DMA as compared to As(V) or As(III) (Abedin et al. 2002; Abbas and Meharg 2008), the movement within the plants is considerably greater than the inorganic As species (Marin et al. 1992; Raab et al. 2007; Carey et al. 2010, 2011).

Carey et al. (2010) using X-ray absorption near edge spectroscopy and inductively coupled plasma mass spectroscopy (ICP-MS) demonstrated that xylem and phloem contribute to As unloading in the rice grain. It has been suggested that for the distribution of As in various plant parts and its speciation phloem is an essential prerequisite (Carey et al. 2010; Tiwari et al. 2014). However, molecular components involved in the transportation of As through phloem are not known till date (Tiwari et al. 2014; Kumar et al. 2015).

7.4 Impact of Arsenic Toxicity on Plant Growth and Physiology

Exposure to As decreases the germination rate, root, and shoot length, causing chlorosis, increased sterility, and ultimately death in a variety of plant species (Liu et al. 2005; Shri et al. 2009; Smith et al. 2010a). In most of the plants, the limit for As toxicity is 5-20 mg/kg (Mendez and Maier 2008). Roots are the first point of contact with As in the soil; hence, they show higher sensitivity towards As toxicity. With increase in the concentration of As, the seedling growth has been shown to be inhibited in rice plants (Shri et al. 2009). Wheat plants when exposed to high levels of As (≥ 80 mg/kg) exhibited decrease in biomass and height of the plant (Gulz et al. 2005). Reduction in the shoot and root system of tomato plants at high As concentration has been observed (Miteva 2002). In *Lens culinaris*, decline in plant height, biomass production, root length, and leaf number was seen with increasing the concentration of As in irrigated water (Ahmed et al. 2006). The reproductive development of a plant exposed to As is also greatly affected (Smith and Read 2008). Abnormal another development, fertilization, inhibition of gametogenesis and sporogenesis, and disruption of female gametophyte development have been reported due to As exposure in plants (Spagnoletti and Lavado 2015).

Arsenic stress damages the chloroplast membrane affecting chlorophyll synthesis in plants (Stoeva and Bineva 2003). Activity of protochlorophyllide reductase is inhibited (Stoeva and Bineva 2003); on entering the leaves, As combines with sulfhydryl ($-SH$) groups of proteins substituting for ferrous (Fe^{2+}), zinc (Zn^{2+}) ions, and destroying the structure of chloroplast in plants (Li et al. 2007). Low content of large subunit of Rubisco was observed in rice leaves (Andrade et al. 2015). Plasmid DNA encodes large subunit of Rubisco, suggesting that As obstructs chloroplast DNA gene expression (Andrade et al. 2015). In the presence of As stress, the chlorophyll fluorescence Fv/Fm ratio that estimates primary photochemistry ability in photosystem II is also affected (Schreiber et al. 1998). Due to As stress, there is damage to the carbon dioxide (CO_2) fixation process resulting in increased internal CO_2 concentration in sub-stomatal spaces (Andrade et al. 2015). Reduction in ATP and NADPH production that are required for CO_2 fixation reactions in calvin-melvin cycle is caused due to As toxicity, therefore hampering photosynthetic electron transport process in plants (Finnegan and Chen 2012; Andrade et al. 2015). A factor which is linked with heat dissipation in plants is non-photochemical quenching (NPQ). This in the presence of As(III) and As(V) increases, indicating oxidative damage and photo-inhibition of the chloroplast (Andrade et al. 2015). Due to substitution of As(V) instead of Pi in ATP, there is formation of ADP-As which is highly unstable compound (Meharg and Macnair 1994). This results in generation of wasteful reaction cycles that leads to uncoupling of photosynthetic electron transport in thylakoid membrane and respiratory electron transport in inner mitochondrial membrane (Avron and Jagendorf 1959).

7.5 Interaction with Phosphorus Uptake

Due to similar chemical properties and electronic configuration, As(V) and P compete for the same uptake carriers in root plasma membrane (Meharg and Macnair 1992; Hartley-Whitaker et al. 2001; Gunes et al. 2009). Therefore, it is imperative to understand their interactions with each other for ascertaining their uptake pathways in plants (Gunes et al. 2009). Plants take up As(V) with the help of P transporters and As(III) in a P independent manner (Wang et al. 2002). Arsenic replaces P in various metabolic pathways; for instance, it competes with P for binding to ATP, resulting in the formation of adenosine diphosphate-As(V) (Trotta et al. 2006). In comparison to As, P binds more efficiently to high-affinity P transporters; thus, high concentration of P in soil solution favors uptake of P than As (Meharg and Macnair 1994; Tu and Ma 2003), As is expelled from the soil solution, and there is increase in P absorption (Alam et al. 2001). Moreover, phosphate is more stable than As(V) over a wide range of pH conditions in soil (Lambkin and Alloway 2003). In a hydroponics study carried out on As hyperaccumulator plants, it was found that in the presence of P the uptake of As in plants is suppressed (Tu and Ma 2003).

7.6 Oxidative Damage

Exposure to As results in the production of reactive oxygen species (ROS) that are formed due to valence changes in metal from As(V) to As(III) (Meharg and Hartley-Whitaker 2002). If in a plant the ROS scavenging system does not work efficiently in the presence of As stress causing formation of free radicals, it leads to uncontrolled oxidation, which results in oxidative stress in plants (Srivastava et al. 2005). The ROS include hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^\bullet), and superoxide radicals ($O_2^{\bullet-}$) (Gunes et al. 2009). These are strong oxidizing agents that result in oxidative damage to biomolecules eventually leading to cell death in plants (Gunes et al. 2009). High levels of As cause lipid peroxidation due to increase in H_2O_2 levels resulting in OH^\bullet formation that cause membrane damage in plants. Presence of As results in increased production of malondialdehyde (MDA; biomarker for lipid peroxidation), low shoot growth, and decrease in dry shoot mass (Gunes et al. 2009) in plants. Increase in the level of MDA has also been observed in hyperaccumulator fern species (Srivastava et al. 2005) and in bean (Stoeva et al. 2005). Severe lipid peroxidation in the presence of As occurs due to hydrogen removal from unsaturated fatty acids by ROS resulting in lipid radical production (Garg and Kaur 2013). Due to this, a cascade of cyclic reactions occur that form short chain-like alkanes and lipid acid aldehydes that damage the lipid structure severely (Mishra et al. 2006; Garg and Kaur 2013). Due to As stress in plants, there is increased amount of energy level in the thylakoids surpassing the level that can be dissipated by metabolic pathways of chloroplast; this disrupts the

electron transport process (Stoeva and Bineva 2003; Stoeva et al. 2004). High lipid peroxidation, chloroplast damage, and decreased protein concentration have been observed in *Pteris ensiformis* on exposure to As (Singh et al. 2006). Increase in thiobarbituric acid (TBA) derivatives, which are indicators of oxidative damage due to As exposure, was found in *P. ensiformis* and in white lupine plants (Singh et al. 2006).

Plants have devised various mechanisms in order to combat oxidative stress. These mechanisms are characterized by induction of various enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) and non-enzymatic antioxidants such as ascorbate, glutathione (GSH), and α -tocopherols (Sairam et al. 2005). By conjugation of $-SH$ groups with the electrophile As or by providing substrates for synthesis of PCs (Gupta et al. 2009), GSH protects plants from damage caused due to As and other metalloids (Schulz et al. 2008). In chickpea plants, activity of CAT and APX has been shown to increase, but is insufficient to remove additional H_2O_2 formed (Gunes et al. 2009). Decrease in the activity of SOD has been observed in various plant species on exposure to As, especially in maize where expression of SOD genes was initially increased at low levels and ultimately decrease on exposure to high levels of As (Mylona et al. 1998). Concentration of non-enzymatic antioxidants decreases in the presence of As (Hartley-Whitaker et al. 2001). In plants, overproduction of proline acts as a protective mechanism towards As stress (Bohnert et al. 1995).

7.7 Phosphorus Uptake Pathways in Plants

7.7.1 Direct Uptake Pathway

The direct uptake pathway of orthophosphate in plants is prevalent in the region behind the root apex (Smith and Smith 2011). In this pathway, Pi is absorbed from the rhizosphere with the help of transporters located in epidermis and the root hair close to the surface of the root. This results in the development of a depletion zone in the rhizosphere (Fig. 7.1) due to faster uptake of Pi and its slow replacement by diffusion from the bulk soil (Gordon-Weeks et al. 2003). It is seen that in the epidermis and root hair cells, there is increase in the expression of genes implicated in encoding high affinity Pi transporters (PiTs) (Gordon-Weeks et al. 2003). This increased gene expression is not seen in case of mature regions of the root probably due to loss of root hair, depletion of Pi in the rhizosphere, and reduced activity of PiTs in the epidermis.

7.7.2 *Indirect/Mycorrhizal Uptake Pathway*

AMF on colonization induce the indirect/mycorrhizal uptake pathway in plants. This pathway overcomes the limitation of direct pathway, and helps in improving plant P nutrition by overcoming the effect of reduced Pi uptake due to the development of depletion zone (Smith and Read 2008). This pathway operates in the region behind the root apex, where direct pathway does not operate (Schnepf et al. 2011). AM fungi form a well-built hyphal network that extends beyond the depletion zone (Fig. 7.1) (Drew et al. 2003). There is deactivation of direct pathway in plants colonized by AMF, due to downregulation of plant PiTs in root epidermis and root hair cells or due to competition for P uptake between root and the hyphal cells in the depletion zone around the root (Schnepf et al. 2008). Plant Pi transporters induced in the presence of AMF has not been described fully. No evidence is available towards the preference of these transporters for Pi or As(V) (Smith et al. 2010a). It has been demonstrated that AM uptake pathway is the dominant route for P uptake even in those plants that do not grow well when colonized by AMF (Jakobsen 1999).

7.7.3 *Adaptations in AMF for Increased Phosphorus Uptake Under As Toxicity*

AMF absorb P even beyond the depletion zone of the rhizosphere, translocating it rapidly to the fungal structures within the roots, and delivering it to the plant root resulting in positive effects on plant growth and biomass (Smith and Read 1997). In AMF, inorganic absorbed P is stored in the form of soluble orthophosphate, polyphosphate granules (Chilvers and Harley 1980), or as soluble polyphosphate. The concentration of inorganic P inside the fungal hyphae has been found to be 1000 times greater than the concentration in soil solution (Gianinazzi-Pearson and Gianinazzi 1986).

Together with the use of $^{33}/^{32}\text{P}$, contribution of AMF pathway in uptake and transfer of Pi and As(V) could be elucidated (Smith et al. 2010b). By using isotopically labeled P supplied to external mycelium, contribution of AMF in P uptake has been observed in *Hordeum vulgare* (Zhu et al. 2003), *Triticum aestivum*, and few of the AMF non-responsive plants like *Lycopersicon esculentum*. In *Medicago truncatula* (medic) and *Linum usitatissimum* (flax) that show high rate of colonization by the fungus, upto 100% of the P was taken up by AMF pathway (Glassop et al. 2005; Smith and Read 2008). In *H. vulgare*, there was decreased expression of epidermal Pi transporters (*HvPht 1;1* and *HvPht 1;2*) implicated in uptake of As(V) and Pi (Christophersen et al. 2009). In AM plants, increased expression of AM-induced transporter (*HvPht 1;8*) was seen, which is involved in increased Pi uptake by roots thereby activating the mycorrhizal uptake pathway; *HvPht 1;8* expression was unaffected by presence of As (Christophersen et al.

2009). AMF inoculated *M. truncatula* have shown high expression of AM inducible Pi transporter *MtPht 1:4*, leading to enhanced P uptake (Christophersen et al. 2012). Hyphal coils of AMF located intracellularly have been associated in P allocation from fungus to the plant, supported by localized expression of *SORTu*; *Pht1;3* related with coils formed by *Gigaspora margarita* in roots of potato (Karandashov and Bucher 2005; Glassop et al. 2005).

7.8 Effect of As Toxicity on AMF Colonization

It has been shown by various studies that plants inoculated with AMF show increased tolerance towards As toxicity, resulting in improved growth and development (Chen et al. 2007; Smith et al. 2010a; Andrade et al. 2015). Few studies on pure culture of AMF suggest that the magnitude of As(V) toxicity vary among fungal taxa (Spagnoletti and Lavado 2015). Effect of AMF on As remediation in plants depends on various isolates of AMF and species of As (Leyval et al. 1997; Orłowska et al. 2005). Under enhanced metal toxicity, growth of AMF can be entirely inhibited (Weissenhorn et al. 1995). It has been observed that in soils with high contamination of HMs such as As, there is decrease in spore number of AMF species, whereas in moderately As contaminated sites there is increase in diversity and species richness of AMF (Del Val et al. 1999). It has been ascertained by morphological spore identification that there is reduction of AMF species diversity in plants growing in As contaminated sites (Karimi et al. 2011). For instance, Andrade et al. (2015) observed decreased intraradical AMF colonization in the presence of As(III) and As(V) in rice plants. In *H. vulgare* inoculated with AMF and grown on metal spiked soil containing As, Cd, Ni, etc., the sporulation capacity of AMF was greatly affected (Biro et al. 2005).

With increase in As content, there is also increase in mycorrhizal colonization in some plants (Hildebrandt et al. 1999; Audet and Charest 2007). Despite having detrimental effects on growth of AMF in As contaminated sites, there have been instances where AMF is known to grow despite As presence. In *Tagetes erecta*, *Melastoma malabathricum*, and *Pityrogramma calomelanos* inoculated with AMF in the presence of As, there has been successful colonization by the fungus (Jankong and Visoottiviseth 2008). Also, there was increase in fungal structures such as vesicles and arbuscules present in these plants upon colonization. Irrespective of As presence, roots of *M. sativa* have shown extensive colonization by *Glomus mosseae* (Chen et al. 2007). In the presence of As (0, 25, 100 mg/kg) and P (25, 100 mg/kg), increase in hyphal length densities in plants inoculated with AMF have been observed (Chen et al. 2007).

AM fungal strains isolated from As contaminated sites have shown high tolerance towards As resulting in improved growth (Vivas et al. 2003; Bai et al. 2008). Indigenous AM fungi (*Glomus* spp, *Acaulospora* spp) have shown high tolerance towards As by resulting in high levels of AM colonization (Bai et al. 2008). For instance, mine AMF isolates of *Glomus* Spp have been shown to be As resistant as

compared to non-mine isolates (Gonzalez-Chavez et al. 2002). AMF isolate *Rhizophagus intraradices* Br1 growing indigenously on metal contaminated sites confers As tolerance on a variety of plant species in diverse environments and was more effective in transferring As tolerance to maize and tomato (Hildebrandt et al. 2007). Biodiversity of AMF in sites contaminated with As has been analyzed by sequencing the nuclear small subunit ribosomal RNA (SSU rRNA) gene fragments using 454-pyrosequencing (Sun et al. 2016). Of all the AMF genera found, *Glomus* was dominant in the mining area. Due to high sporulation rate, *Glomus* spp show better adaptation in sites contaminated with As and hence resulting in improved ability to recover from As toxicity (Whitfield et al. 2004; Daniell et al. 2001).

7.9 Mechanisms Underlying Alleviation of As Toxicity by AMF Colonization

7.9.1 Enhanced Phosphorus Uptake and Biomass

As AMF assist plant uptake of P, it is essential to consider P–As interactions for ascertaining the role of AMF in As uptake in plants (Zhang et al. 2015). When inoculated with AMF, in the presence of As in soil, there is downregulation of P as well as As uptake by direct pathway, whereas there is enhanced expression of indirect pathway which shows selectivity towards P uptake in plants (Glassop et al. 2005), resulting in a higher P/As ratio (Fig. 7.2) (Adriano 2001; Ahmed et al. 2006; Chen et al. 2007; Ultra et al. 2007). Reduction in As translocation factor (TF) and high P/As ratio were seen in *Melastoma malabathricum*, preventing As translocation to the aerial plant parts (Jankong and Visoottiviseth 2008). Therefore, AMF alleviate As toxicity by improving P nutrition and reducing As aggregation to the shoots in plants (Chen et al. 2007). In *Leucaena leucocephala* inoculated with *G. clarum*, there occurs decrease in As translocation factor (<0.99), suggesting sequestration of As in roots of the plants (Xu et al. 2008; Schneider et al. 2013). It has been shown that by inoculation with *G. geosporum*, there is increase in yield of rice plants grown in As contaminated sites (Li et al. 2011b). This increase occurs due to enhanced P/As ratio and decreased grain/straw As content in AMF inoculated plants, resulting in translocation of As from grain to straw mediated by P transporters (Li et al. 2011b).

The enhanced biomass of plant due to AMF colonization accounts for the decreased internal As concentrations, resulting in “dilution effect” observed in mycorrhizal plants (Chen et al. 2007). AMF inoculation enhances P nutrition and plant biomass under As stress and a relative As dilution because P shares chemical properties with As. In *M. malabathricum* plants grown in arsenic (As) contaminated soils, inoculation with AMF resulted in increased surface area of the roots allowing better growth and development of the plant (Jankong and Visoottiviseth 2008). *M. truncatula* and *Allium porrum* when inoculated with a mixture of AMF species

accumulated more P and maintained greater plant growth and development (Jansa et al. 2008). By increasing the P uptake in plants, AMF cause dilution of As (Ahmed et al. 2006; Ultra et al. 2007). It is believed that due to the presence of efficient P uptake mechanism in AMF with high selectivity towards P as compared to that of As, there is increase in the uptake of P in plants (Smith et al. 2010b). In lentil (*L. culinaris*) plants inoculated with *G. mosseae*, and irrigated with 1, 2, 5, and 10 mg As(V) L⁻¹, decreased As concentration was found in the pods leading to diminishing As toxicity caused due to consumption of contamination food grains (Ahmed et al. 2006). It has been found that at high concentration of As in soil, maize plants treated with indigenously growing AMF (*Glomus* spp, *Acaulospora* spp.) from As contaminated sites exhibited higher biomass due to enhanced root P as that of non-mycorrhizal plants (Bai et al. 2008). Mycorrhiza growing indigenously has shown to sustain growth of *Pteris vittata* plants by aiding P absorption in As polluted soil (Leung et al. 2006, 2013). Biomass of *G. mosseae* was not influenced by high content of As in soil (200 mg/kg) (Xu et al. 2008). Instead due to high P nutrition resulting from AM, there was increase in shoot biomass and decrease in root As concentration (Xu et al. 2008).

7.9.2 Biotransformation of As

Inoculation with AMF influences speciation and transformation of As in host plant (Zhang et al. 2015). Transformation of As(V) into less toxic organic forms is also one of the strategies employed by AMF to ameliorate As toxicity (Gonzalez-Chavez et al. 2002; Ultra et al. 2007; Jia et al. 2012). Plants do not have the ability to methylate As species; therefore, methylation of As species is carried out by soil microorganisms (Lomax et al. 2012). AMF by releasing substrates in the rhizosphere activate microorganisms that assist in bio-methylation of As in plants (Mukhopadhyay et al. 2002). Presence of methylated As forms has been observed in rhizosphere of *Helianthus annuus* on inoculation with *Glomus aggregatum* (Ultra et al. 2007). Elevated content of DMA in rice grains on inoculation with *R. intraradices* has been reported (Li et al. 2016). Gonzalez-Chavez Mdel et al. (2011) found that high affinity phosphate transporters present on the extraradical mycelium of AMF such as GiPT in *R. intraradices* are responsible for the uptake of As(V) into the fungal mycelium where it gets transformed to As(III) by arsenate reductases. In order to prevent the intracellular toxicity caused by As(III) in the fungal hyphae, a membrane bound As(III) efflux pump is activated (GiArsA/B) (for *Glomus intraradices*) (Liu et al. 2003). This As(III) efflux pump pumps out As(III) in the surrounding medium. Analogous mechanisms of As(V) reduction and efflux of As(III) in the surrounding have been reported in tomato and rice (Xu et al. 2007). GvArsA (for *Glomus versiformis*) and GiArsA have been shown to have higher protein sequence similarity with each other. In GvArsA, there is presence of conserved domains such as ATP binding site, metal binding site, and dimerization interface similar to that seen in ArsA ATPases (Ye et al. 2010; Yang et al. 2011). In

bacteria on the inner membrane surface is present ArsA ATPase that functions as efflux pumps (Shen et al. 2003). ArsA ATPases are implicated in transport of As (III) and Sb (III) (Antimony) across membranes in bacteria (Ye et al. 2010). In *Caenorhabditis elegans*, a functional ArsA ATPase, ASNA1, shows a high degree of homology with GvArsA (Tseng et al. 2007). These observations support the concept that GvArsA is involved as one of the components of *G. versiformis* efflux As(III) pump (Gonzalez-Chavez Mdel et al. 2011, 2014). Localization of GiArsA protein is seen in plasma membrane similar to that of bacterial ArsAB ATPase, suggesting that As (III) might be effluxed to periarbuscular space (Gonzalez-Chavez Mdel et al. 2011).

7.9.3 Reduced As Uptake

Decreased uptake of As from soil is one of the strategies employed by AMF to combat As toxicity. Gonzalez-Chavez et al. (2002) suggested that regardless of the plant host genotype for As(V) tolerance, all the AMF strains confer additional As tolerance to the plants. They affirmed that As(V) influx was reduced in plant roots by the suppression of high-affinity As(V)/Pi transporters. It has been found that in *Holcus lanatus* plants inoculated with AMF, there was reduction in As(V) influx due to suppression of As(V)/Pi transporters in plant roots, therefore decreasing As (V) uptake (Gonzalez-Chavez et al. 2002). Mycorrhizal inoculation remarkably inhibited As(V) uptake in rice in short-term affinity uptake system (Li et al. 2011b). Uptake of As(III) and MMA was also lowered in both high and low-affinity transport system in plants inoculated with AMF (Li et al. 2011a). The mechanism of As uptake and localization of As in AMF is not yet known. By employing radioactive $^{73}\text{As(V)}$, the fungal potential for transport and uptake of As could be known (Smith et al. 2010a).

Zhang et al. (2014) examined the influence of AMF inoculation on accumulation of As and its speciation in *M. truncatula*. They reported that while specific Pi uptake was augmented by AMF colonization, As uptake was decreased. AMF mediated decreased influx of As species is due to reduced expression of phosphate transporters such as *OsPHT2* and silicon transporters, *Lsi1* and *Lsi2*, thus resulting in reduced uptake of As(V) and As(III), respectively (Ma et al. 2008). *Lsi1* is a member of nodulin 26-like intrinsic membrane proteins (NIPs). They help in the transport of silicon (Si) from external medium into rice roots (Ma et al. 2008). They also help in the transfer of As(III) from soil into the rice roots (Li et al. 2009). Silicic acid and As(III) compete for transportation in the rice roots. AMF may suppress the expression of *Lsi1* to limit the uptake of As (Chen et al. 2015).

7.9.4 Sequestration of As

Following the uptake of metal from the soil, AMF sequester it in various fungal structures such as vesicles and intra- and extraradical hyphae (Christie et al. 2004; Wang et al. 2007). In order to prevent the entry of metal in the cytoplasm, As is stored in the vesicular structures of AMF (Gohre and Paszkowski 2006). It could also be the case wherein the toxic compound is changed into less toxic forms and transported to plants or effluxed into the surrounding medium (Meharg 2003). For instance, conversion of As(V) to As(III). Also metal chelators such as amino acids, phytochelatins, and metallothioneins may have a role in transport and storage of toxic metal ions (Meharg 2003). AMF due to the presence of chitin in their cell walls have the ability to bind and immobilize As (Gaur and Adholeya 2004). The fungal cell walls also possess various free hydroxyl, amine, and imidazole carboxyl groups which offer active sites for binding As and prevent their movement in plants (Joiner et al. 2000). Due to intracellular precipitation of metallic cations with phosphate, there is decreased translocation of metals to the plants (Turnau et al. 1993).

7.9.4.1 Production of Glomalin and Organic Acids

Glomalin is a glycoprotein produced by AMF that trigger soil formation (Rillig and Mummey 2006). During enhanced fungal growth, glomalin accumulates outside extraradical mycelium (Wright and Upadhyaya 1996). By forming complexes with various heavy metals including As, production of glomalin reduces As uptake in plants (Gaur and Adholeya 2004). This glycoprotein is also rich in iron; therefore, it may be involved in detoxification by forming As(III)-Fe(III) oxide compounds (Chen et al. 2005). Iron oxides when present have greater affinity for As(V) and As(III) (Meng et al. 2002). Role of glomalin in As tolerance has been known in various wetland plants (Meng et al. 2002).

AMF by producing organic acids help to mobilize elements essential for plant growth and development. Organic acids such as oxalic, malic, and citric acids are produced by the fungus (Jones 1998). For instance, in pine AMF colonize roots and result in greater production of oxalic acid as compared to that of non-colonized roots (Meharg 2003). Organic acids released by mycorrhiza have the ability to act as methyl donors resulting in bio-methylation and production of substrates that enhance microbial activity (Mukhopadhyay et al. 2002). These organic acids help to mobilize P ions from insoluble and complexed iron and aluminium phosphates in the rhizosphere (Ahonen-Jonnarth et al. 2000). Whereas, toxic metals present in the soil are immobilized by precipitation with organic acids (Meharg 2003). However, cycling and ability of organic acids to immobilize metals is influenced by their affinity for a particular metal (Meharg 2003).

7.9.4.2 Change in Soil pH

Effect of soil pH on community composition of AMF is significant (Meharg 2003; Xiang et al. 2014). Soil pH of mycorrhizosphere influences spore density of the AMF species (Tchabi et al. 2008; Sun et al. 2016). Extent of AMF colonization and the community composition is influenced by hyphal growth, formation, and spore germination (Robson and Abbott 1989; Coughlan et al. 2000) which in turn is controlled by the pH of the soil. Some genera of AMF prefer acidic soils, while some others are present in a broader range of pH (Maia and Trufem 1990). For instance, AMF genus *Glomus* prefers neutral or alkaline pH (Schenck and Siqueira 1987) whereas *Gigaspora*, *Entrophospora*, and *Sclerocystis* prefer acidic soils (da Silva et al. 2005). At low pH, build up of positive charge occurs that repel cations resulting in metal cation insensitivity (Green et al. 1976). Inoculation with mycorrhizza results in reduction of pH in the vicinity of rhizosphere due to selective uptake of nutrients (Smith and Read 1997; Sun et al. 2016).

7.9.4.3 Higher Production of Phytochelatins and Metallothioneins

Arsenic chelation in the cytosol by high affinity ligands is potentially a very important mechanism of As detoxification and tolerance in plants under As stress. Two types of peptide metal binding ligands are synthesized by plants: phytochelatins (PCs) and metallothioneins (MTs). Phytochelatins are a family of cysteine-rich polypeptides, which play important role in detoxification of many HMs. Phytochelatin synthase (PCS) catalyzes the formation of phytochelatins from glutathione. Glutathione is known to play a central role in antioxidant defense system by upregulating cysteine synthase (CS), GST, and GR. Arsenate [As(V)] can be readily reduced to arsenite [As(III)] via arsenate reductase (ACR) enzyme (Mukhopadhyay et al. 2000; Smith et al. 2010a), which then subsequently complexed with thiols, particularly PCs. It has been proposed that PC-As complexes can be sequestered into the vacuoles by yef1p (ABC-type transporter) transporters and confers As(III) resistance in yeast (Ghosh et al. 1999; Zhu and Rosen 2009). Christophersen et al. (2012) confirmed that both *R. intraradices* and *Funneliformis mosseae* inoculation resulted in significantly higher expressions of *MtPCS* (*M. truncatula*) compared with non-mycorrhizal plants under As stress. They also reported increased expression of arsenate reductase (*MtACR*) gene that codes proteins thought to be involved in arsenate detoxification. Cicatelli et al. (2010) showed higher MTs gene expression in AMF inoculated plants of *Populus alba* clone AL35, suggesting that these polypeptides may provide protection from HM-induced toxicity. Rivera-Becerril et al. (2005) also reported similar results in *Pisum sativum*. Metallothioneins probably exert an antioxidant function (Akashi et al. 2004). Although role and production of PCs and MTs have been studied in many metals, not much is known about the effect of these protein ligands in response to AMF inoculation under As stress.

7.10 Amelioration of As Toxicity on Plant Physiology

The process of photosynthesis is stimulated due to improved nutrition in mycorrhizal plants (Dong et al. 2008). Total chlorophyll content was increased in *Solanum melongena* plants on inoculation with *G. mosseae* (Aziz et al. 2011). In maize leaves, AMF resulted in enhanced photochemical, non-photochemical efficiencies, and gas exchange, resulting in increased photosynthesis of plants (Sheng et al. 2008). Plants inoculated with AMF show increased nitrogen content as compared to that of non-colonized plants (Andrade et al. 2015). Colonization by AMF induces calvin–melvin cycle in plants (Sheng et al. 2008). This is done so as to increase the transport of triose phosphates to the roots, in order to reduce its limitation on photosynthesis resulting in increased carbon dioxide fixation in leaves (Kaschuk et al. 2009).

Comparative analysis of proteins induced in the presence of As contamination and mycorrhizal inoculation in *P. vittata* plants have shown upregulation of glycolytic enzymes (Bona et al. 2011). Glyceraldehyde-3-phosphate dehydrogenase (GADPH) catalyzes the formation of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate and phosphate in plants. GADPH can use As(V) instead of phosphate converting glyceraldehyde 3-phosphate into 1-arseno-3-phosphoglycerate (Gregus et al. 2009). One isoform of GADPH has been shown to increase in the presence of AMF (Bona et al. 2010). All enzymes that catalyze phosphorolytic–arsenolytic processes readily convert arsenylated metabolites to As(III) in plants (Bona et al. 2011). In plant roots colonized by *G. mosseae*, there is increase in phenylalanine-tRNA ligase or phenylalanyl-tRNA synthetase indicating the induction of protein synthesis in the presence of As (Zhou et al. 2010).

7.11 Protection from Oxidative Damage

In plants inoculated with AMF, increase in the concentration of glutathione (GSH) has been observed, suggesting glutathionylation as an approach for As detoxification in plants (Bona et al. 2011). High GSH pool in plants increases the tolerance of plants towards As toxicity. High GSH level was found in fronds of *P. vittata* where it could participate in the reduction of As(V) to As(III) in vitro (Singh et al. 2006). Increase in GSH levels have been reported on inoculation of plants with AMF (Garg and Kaur 2013). In AMF inoculated plants, increase in gene expression of GR has been observed that provides protection from antioxidants by recycling glutathione from oxidized to reduced form (Fuentes et al. 2016). In order to sustain increased ratio of GSH/GSSG, production of GSH is essential as it is involved in the synthesis of PCs and other enzymes of ROS scavenging pathway (Garg and Kaur 2013). Garg and Singla (2012) reported low levels of H₂O₂ and MDA in pea plants inoculated with AMF and grown on 30, 60, and 90 mg/kg As contaminated soil. Lipid peroxidation in plants can be ascertained by estimating the amount of thiobarbituric

acid (TBA) formed (Gonzalez-Chavez et al. 2002). On inoculation of soybean plants with *R. intraradices* at a dose of 25 or 50 mg/kg As, there was observed decrease in the content of TBA in both leaves and roots as compared to non-inoculated soybean plants (Spagnoletti et al. 2016). This decrease in lipid peroxidation on inoculation with AMF is attributed to reduced production of ROS resulting in reduced oxidative damage in plants (Garg and Kaur 2013; Fuentes et al. 2016).

Mycorrhizal plants in the presence of As show increase in the production of SOD, CAT, and APX (Wu et al. 2010; Garg and Singla 2012). Increase in concentration of ascorbate, glutathione, non-protein thiols, and cysteine has also been observed in plants treated with As (Mishra et al. 2008). Reduction in ROS production and lipid peroxidation occurs due to inoculation with AMF (Rahmaty and Khara 2008). In AMF colonized plants, high level of peroxidase (POD) is indicative of lower ROS production as compared to non-colonized plants (Santana et al. 2015). There is increase in the production of proline, glycine betaine, total proteins, and soluble sugars in pea (*P. sativum*) plants inoculated with *G. mosseae* when grown under As(V) stress (Garg and Singla 2012). Buildup of proline rich proteins provides protection against As stress (Matysik et al. 2002). Proline reduces As toxicity in plants by chelation of this HM in the cytoplasm (Schat et al. 1997), reducing hydroxyl radical production (Smirnoff and Cumbes 1989) and decreasing uptake of metal.

7.12 Use of Hyperaccumulators in Conjunction with AMF

Several species of fern from the genus *Pteris* are able to accumulate extremely high concentrations of arsenic (As) in the fronds. *P. vittata*, *P. cretica*, and *P. biaurita* are the well-known hyperaccumulators of As and are able to accumulate huge quantities of As from the soil (Ma et al. 2001). These hyperaccumulators show increased tolerance when grown in As contaminated soils in the presence of mycorrhiza (Leung et al. 2006). When supplied with AMF in the presence of As, *P. vittata* exhibited enhanced frond surface area and improved leaf area (Trotta et al. 2006). These parameters contribute to increase in As accumulating capacity of the fern as *P. vittata* accumulates As in the pinnae epidermis. Higher As translocation factor leading to high As storing ability was seen in the fern.

In the frond epidermal cells, majority of As is sequestered in un-complexed form in the vacuole. When As uptake exceeds the vacuolar sequestration capacity, PCs help in detoxification process in the fern (Zhao et al. 2003; Trotta et al. 2006). It has been found that under As stress, glutathione concentration is increased in fern roots and fronds which is a substrate for phytochelatin synthesis (Bona et al. 2011). Proteomic study of *P. vittata* suggested that As modulates the levels of numerous proteins related to glycolysis (Bona et al. 2011). Another study revealed that under As stress, a member of ABC transporter family, PDR-like protein is increased in *P. vittata* and helps in the detoxification of As (Shen et al. 2014). Mycorrhiza also

improves P nutrition lowering As toxicity by favoring the Pi uptake via AM pathway. Higher activity of arsenate reductase has been observed in *P. vittata* inoculated with mycorrhiza (Leung et al. 2013). In *P. vittata* inoculated with *G. mosseae* and *Gigaspora margarita*, there was upregulation of PgPOR29, porins that facilitate passive transport of small sized molecules in the presence of As (Bona et al. 2010). PgPOR29 may be involved in conferring As resistance in fern by either increasing As uptake or by causing As efflux and sequestration in the vacuoles of the frond (Bona et al. 2010).

7.13 Conclusion

Biological methods including the use of soil microbes and plants are amongst the most suitable methods, environmentally and economically. Use of AMF in alleviation of As stress is a reliable and efficient approach. Inoculation with AMF has shown to increase the yield of plants without increasing the concentration of As. AM fungi are known to absorb slight amounts of As as well. The absorbed As is retained in the fungal compartment preventing its translocation to roots and subsequently to the shoots. Ability of AMF to take up As strongly depends on the fungal isolate being used. It has been validated by various studies that best adapted fungal isolates for alleviating toxicity of As contaminated soils are the ones indigenously growing in the polluted soils. Therefore, for desired outcomes, AMF strains should be carefully selected, especially when being used in agriculture or in remediation of contaminated lands. It is also known that a variety of plants have the inbuilt mechanisms and the genetic potential to clean sites contaminated with As. Molecular understanding of As hyperaccumulators when used in conjunction with AMF is limited. Therefore, identification of As hyperaccumulator plants and their usage in conjunction with AMF can prove to be a beneficial technology. Understanding the physical, chemical, and biological mechanistic basis of the tripartite interaction between As, AMF, and plants will enable us to strategize efficiently phytoremediation of As contaminated land and rescuing of crop plants.

References

- Abbas MHH, Meharg AA (2008) Arsenate, arsenite and dimethyl arsenic acid (DMA) uptake and tolerance in maize (*Zea mays* L.) Plant Soil 304:277–289
- Abedin MJ, Cresser MS, Meharg AA et al (2002) Arsenic accumulation and metabolism in rice. Environ Sci Technol 36:962–968
- Acharya SK (2002) Arsenic contamination in groundwater affecting major parts of southern West Bengal and parts of western Chattisgarh: source and mobilization processes. Curr Sci 82:740–744
- Adriano DC (2001) Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals, 2nd edn. Springer, New York

- Ahmed FRS, Killham K, Alexander I (2006) Influences of arbuscular mycorrhizal fungus *Glomus mosseae* on growth and nutrition of lentil irrigated with arsenic contaminated water. *Plant Soil* 258:33–41
- Ahonen-Jonnarh U, van Vees PAW, Lundstrom US, Finlay RD (2000) Organic acids produced by mycorrhizal *Pinus sylvestris* exposed to elevated aluminium and heavy metal concentrations. *New Phytol* 146:557–567
- Akashi K, Nishimura N, Ishida Y et al (2004) Potent hydroxyl radical scavenging activity of drought-induced type-2 metallothionein in wild watermelon. *Biochem Biophys Res Commun* 323:72–78
- Alam MGM, Tokunaga S, Maekawa T (2001) Extraction of arsenic in a synthetic arsenic-contaminated soil using phosphate. *Chemosphere* 43:1035–1041
- Andrade SAL, Domingues AP, Mazzafera P (2015) Photosynthesis is induced in rice plants that associate with arbuscular mycorrhizal fungi and are grown under arsenate and arsenite stress. *Chemosphere* 134:141–149
- Audet P, Charest C (2007) Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: meta-analytical and conceptual perspectives. *Environ Pollut* 147:609–614
- Avron M, Jagendorf A (1959) Evidence concerning the mechanism of adenosine triphosphate formation by spinach chloroplasts. *J Biol Chem* 234:967–972
- Aziz I, Ayoob M, Paramjit K (2011) Response of *Solanum melongena* to inoculation with arbuscular mycorrhizal fungi under low and high phosphorus condition. *Not Sci Biol* 3:70–74
- Bai J, Lin X, Yin R et al (2008) The influence of arbuscular mycorrhizal fungi on As and P uptake by maize (*Zea mays* L.) from As-contaminated soils. *Appl Soil Ecol* 38:137–145
- Bates MN, Smith AH, Cantor KP (1995) Case-control study of bladder cancer and arsenic in drinking water. *Am J Epidemiol* 141:523–530
- Bentley R, Chasteen TG (2002) Microbial methylation of metalloids: arsenic, antimony, and bismuth. *Microbiol Mol Biol Rev* 66:250–271
- Bienert GP, Thorsen M, Schüssler MD et al (2008) A subgroup of plant aquaporins facilitate the bidirectional diffusion of As(OH)₃ and Sb(OH)₃ across membranes. *BMC Biol* 6:26. doi:10.1186/1741-7007-6-26
- Biro B, Posta K, Füzy A et al (2005) Mycorrhizal functioning as part of the survival mechanisms of barley (*Hordeum vulgare* L.) at long-term heavy metal stress. *Acta Biol Szegedien* 49:65–67
- Bleeker PM, Hakvoort HW, Bliet M et al (2006) Enhanced arsenate reduction by a CDC25-like tyrosine phosphatase explains increased phytochelatin accumulation in arsenate-tolerant *Holcus lanatus*. *Plant J* 45:917–929
- Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptions to environmental stresses. Plant polyamines inhibit lipid peroxidation in senescing oat leaves. *Physiol Plant* 99:385–390
- Bona E, Cattaneo C, Cesaro et al (2010) Proteomic analysis of *Pteris vittata* fronds: two arbuscular mycorrhizal fungi differentially modulate protein expression under arsenic contamination. *Proteomics* 10:3811–3834
- Bona E, Marsanoa F, Massaa N et al (2011) Proteomic analysis as a tool for investigating arsenic stress in *Pteris vittata* roots colonized or not by arbuscular mycorrhizal symbiosis. *J Proteome* 7:1338–1350
- Carey AM, Scheckel KG, Lombi E et al (2010) Grain unloading of arsenic species in rice. *Plant Physiol* 152:309–319
- Carey AM, Norton GJ, Deacon C et al (2011) Phloem transport of arsenic species from flag leaf to grain during grain filling. *New Phytol* 192:87–98
- Catarecha P, Segura MD, Franco-Zorrilla JM et al (2007) A mutant of the *Arabidopsis* phosphate transporter PHT1;1 displays enhanced arsenic accumulation. *Plant Cell* 19:1123–1133
- Cebrian ME, Albores A, Aquilar M et al (1983) Chronic arsenic poisoning in the north of Mexico. *Hum Toxicol* 2:121–133
- Chakraborti D, Singh EJ, Das B et al (2008) Groundwater arsenic contamination in Manipur, one of the seven North-Eastern Hill states of India: a future danger. *Environ Geol* 56:381–390

- Chakraborti D, Rahman MM, Murrill M et al (2013) Environmental arsenic contamination and its health effects in a historic gold mining area of the Mangalur greenstone belt of Northeastern Karnataka, India. *J Hazard Mater* 262:1048–1055
- Chen Z, Zhu YG, Liu WJ et al (2005) Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol* 165:91–97
- Chen B, Xiao X, Zhu YG et al (2007) The arbuscular mycorrhizal fungus *Glomus mosseae* gives contradictory effects on phosphorus and arsenic acquisition by *Medicago sativa* (L.) *Sci Total Environ* 379:226–234
- Chen Y, Moore KL, Miller AJ et al (2015) The role of nodes in arsenic storage and distribution in rice. *J Ext Bot* 66:3717–3724
- Chilvers GA, Harley JL (1980) Visualization of phosphate accumulation in beech mycorrhizas. *New Phytol* 4:319–326
- Christie P, Li X, Chen B (2004) Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. *Plant Soil* 261:209–217
- Christophersen HM, Smith FA, Smith SE (2009) Arbuscular mycorrhizal colonization reduces arsenate uptake in barley via downregulation of transporters in the direct epidermal phosphate uptake pathway. *New Phytol* 184:962–974
- Christophersen HM, Smith FA, Smith SE (2012) Unraveling the influence of arbuscular mycorrhizal colonization on arsenic tolerance in medicago: *Glomus mosseae* is more effective than *G. intraradices*, associated with lower expression of root epidermal Pi transporter genes. *Front Physiol* 3:91. doi:10.3389/fphys.2012.00091
- Cicatelli A, Lingua G, Todeschini V et al (2010) Arbuscular mycorrhizal fungi restore normal growth in a white poplar clone grown on heavy metal-contaminated soil, and this is associated with upregulation of foliar metallothionein and polyamine biosynthetic gene expression. *Ann Bot* 106:91–802
- Coughlan AP, Dalpé Y, Lapointe L et al (2000) Soil pH-induced changes in root colonization, diversity, and reproduction of symbiotic arbuscular mycorrhizal fungi from healthy and declining maple forests. *Can J For Res* 30:1543–1554
- Covey RP, Koch BL, Larsen HJ (1981) Influence of vesicular arbuscular mycorrhizae on the growth of apple and corn in low-phosphorus soil. *Phytopathology* 71:712–715
- Cullen WR, Reimer KJ (1989) Arsenic speciation in the environment. *Chem Rev* 89:713–764
- da Silva GA, Trufem SFB, Júnior OJS et al (2005) Arbuscular mycorrhizal fungi in a semiarid copper mining area in Brazil. *Mycorrhiza* 15:47–53
- Daniell TJ, Husband R, Fitter AH et al (2001) Molecular diversity of arbuscular mycorrhizal fungi colonizing arable crops. *FEMS Microbiol Ecol* 36:203–209
- Das HK, Mitra AK, Sengupta PK et al (2004) Arsenic concentrations in rice, vegetables and fish in Bangladesh: a preliminary study. *Environ Int* 30:383–387
- Del Val C, Barea JM, Azon Aguilar C (1999) Diversity of arbuscular mycorrhizal fungus populations in heavy-metal contaminated soils. *Appl Environ Microbiol* 65:718–723
- Dong Y, Zhu YG, Smith FA et al (2008) Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) plants in an arsenic-contaminated soil. *Environ Pollut* 15:174–181
- Drew EA, Murray RS, Smith SE et al (2003) Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant Soil* 251:105–114
- EPA (1988) Special report on ingested inorganic arsenic: skin cancer; nutritional essentiality. EPA 625/3-87/013 U.S. Environmental Protection Agency, Risk Assessment Forum, Washington
- Finnegan PM, Chen W (2012) Arsenic toxicity: the effects on plant metabolism. *Front Physiol* 3:1–18
- Fuentes A, Almonacid L, Ocampo JA et al (2016) Synergistic interactions between a saprophytic fungal consortium and *Rhizopogon irregularis* alleviate oxidative stress in plants grown in heavy metal contaminated soil. *Plant Soil* 407:355–366

- Garg N, Kaur H (2013) Response of antioxidant enzymes, phytochelatins and glutathione production towards Cd and Zn stresses in *Cajanus cajan* (L.) Millsp. Genotypes colonized by arbuscular mycorrhizal fungi. *J Agron Crop Sci* 9:31–42
- Garg N, Singla P (2011) Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. *Environ Chem Lett* 9:303–321
- Garg N, Singla P (2012) The role of *Glomus mosseae* on key physiological and biochemical parameters of pea plants grown in arsenic contaminated soil. *Sci Hortic* 143:92–101
- Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Curr Sci* 8:528–534
- Ghosh M, Shen J, Rosen BP (1999) Pathway of As(III) detoxification in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 96:5001–5006
- Gianinazzi-Pearson V, Gianinazzi S (1986) The physiology of improved phosphate nutrition in mycorrhizal plants. In: Gianinazzi-Pearson V, Gianinazzi S (eds) *Physiological and genetical aspects of mycorrhizae*. INRA, Paris, pp 101–109
- Glassop D, Smith SE, Smith FW (2005) Cereal phosphate transporter associated with the mycorrhizal pathway of phosphate uptake into roots. *Planta* 222:688–698
- Gohre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* 223:1115–1122
- Gonzalez E, Solano R, Rubio V et al (2005) Phosphate transporter traffic facilitator1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in *Arabidopsis*. *Plant Cell* 17:3500–3512
- Gonzalez-Chavez Mdel C, Ortega-Larrocea MD, Carrillo-Gonzalez R et al (2011) Arsenate induces the expression of fungal genes involved in As transport in arbuscular mycorrhiza. *Fungal Biol* 115:1197–1209
- Gonzalez-Chavez Mdel C, Miller B, Maldonado-Mendoza IE et al (2014) Localization and speciation of arsenic in *Glomus intraradices* by synchrotron radiation spectroscopic analysis. *Fungal Biol* 118:444–452
- Gonzalez-Chavez C, Harris PJ, Dodd J et al (2002) Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. *New Phytol* 15:163–171
- Gordon Weeks R, Tong Y, Davies TGE et al (2003) Restricted spatial expression of a high-affinity phosphate transporter in potato roots. *J Cell Sci* 116:3135–3314
- Green NE, Graham SO, Schenck NC (1976) The influence of pH on the germination of vesicular-arbuscular mycorrhizal spores. *Mycologia* 68:929–934
- Gregus Z, Roos G, Geerlings P et al (2009) Mechanism of thiol-supported arsenate reduction mediated by phosphorolytic–arsenolytic enzymes II. Enzymatic formation of arsenylated products susceptible for reduction to arsenite by thiols. *Toxicol Sci* 110:282–292
- Gulz PA, Gupta SK, Schulin R (2005) Arsenic accumulation of common plants from contaminated soils. *Plant Soil* 272:337–347
- Gunes A, Pilbeam DJ, Inal A (2009) Effect of arsenic-phosphorous interaction on arsenic-induced oxidative stress in chickpea plants. *Plant Soil* 314:211–220
- Gupta M, Sharma P, Sarin NB et al (2009) Differential response of arsenic stress in two varieties of *Brassica juncea* (L.) *Chemosphere* 74:1201–1208
- Hartley-Whitaker J, Ainsworth G, Meharg A (2001) Copper and arsenic induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. *Plant Cell Environ* 24:713–722
- Hasanuzzaman M, Fujita M (2012) Heavy metals in the environment: current status, toxic effects on plants and possible phytoremediation. In: Anjum NA, Pereira PA, Ahmad I, Duarte AC, Umar S, Khan NA (eds) *Phytotechnologies: remediation of environmental contaminants*. Taylor and Francis/CRC Press, USA, pp 8–73
- Hildebrandt U, Kaldorf M, Bothe H (1999) The zinc violet and its colonization by arbuscular mycorrhizal fungi. *J Plant Physiol* 154:709–711
- Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry* 68:139–146

- Hua L, Wu W, Liu Y et al (2009) Reduction of nitrogen loss and Cu and Zn mobility during sludge composting with bamboo charcoal amendment. *Environ Sci Pollut Res* 16:1–9
- Huang JH, Matzner E (2006) Dynamics of organic and inorganic arsenic in the solution phase of an acidic fen in Germany. *Geochim Cosmochim Acta* 70:2023–2033
- Hughes MF (2002) Arsenic toxicity and potential mechanisms of action. *Toxicol Lett* 133:1–6
- Huysman KD, Frankenberger WT (1990) Arsenic resistant microorganisms isolated from agricultural drainage water and evaporation pond sediments. *Water Air Soil Pollut* 53:159–168
- IARC (1980) Monographs on the evaluation of the carcinogenic risk of chemicals to man: some metals and metallic compounds. International Agency for Research on Cancer, Lyon, pp 39–141
- IARC (1987) Monographs on the evaluation of the carcinogenic risk to humans: arsenic and arsenic compounds (Group 1). Supplement 7, International Agency for Research on Cancer, Lyon, pp 100–103
- Jakobsen I (1999) Transport of phosphorus and carbon in arbuscular mycorrhiza. *Mycorrhiza: structure, function*. Mol Biol Biotechnol, 2nd edn. Springer, Berlin
- Jankong P, Visoottiviseth P (2008) Effects of arbuscular mycorrhizal inoculation on plants growing on arsenic contaminated soil. *Chemosphere* 72:1092–1097
- Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytol* 177:779–789
- Jia Y, Huang H, Sun GX et al (2012) Pathways and relative contributions to arsenic volatilization from rice plants and paddy soil. *Environ Sci Technol* 46:8090–8096
- Joiner EJ, Briones R, Leyval C (2000) Metal binding capacity of arbuscular mycorrhizal mycelium. *Plant Soil* 226:227–234
- Jones DL (1998) Organic acids in the rhizosphere – a critical review. *Plant Soil* 205:25–44
- Karandashov V, Bucher M (2005) Symbiotic phosphate transport in arbuscular mycorrhizas. *Trends Plant Sci* 10:22–29
- Karimi A, Khodaverdiloo H, Sepehri M et al (2011) Arbuscular mycorrhizal fungi and heavy metal contaminated soils. *Af J Microbiol Res* 5:1571–1576
- Kaschuk G, Kuyper TW, Leffelaar PA et al (2009) Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biol Biochem* 41:1233–1244
- Kumar S, Dubey RS, Tripathi RD et al (2015) Omics and biotechnology of arsenic stress and detoxification in plants: current updates and prospective. *Environ Int* 74:221–230
- Lambkin DC, Alloway BJ (2003) Arsenate-induced phosphate release from soils and its effect on plant phosphorus. *Water Air Soil Pollut* 144:41–56
- Leung HM, Ye ZH, Wong MH (2006) Interactions of mycorrhizal fungi with *Pteris vittata* (As hyperaccumulator) in As-contaminated soils. *Environ Pollut* 139:1–8
- Leung HM, Leung AOW, Ye ZH et al (2013) Mixed arbuscular mycorrhizal (AM) fungal application to improve growth and arsenic accumulation of *Pteris vittata* (As hyperaccumulator) grown in As-contaminated soil. *Chemosphere* 92:1367–1374
- Leyval C, Turnan K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7:139–153
- Li Z, Beachner R, Mc Manama Z et al (2007) Sorption of arsenic by surfactant modified zeolite and kaolinite. *Microporous Mesoporous Mater* 105:291–297
- Li RY, Ago Y, Liu WJ et al (2009) The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. *Plant Physiol* 150:2071–2080
- Li H, Wu C, Ye ZH et al (2011a) Uptake kinetics of different arsenic species in lowland and upland rice colonized with *Glomus intraradices*. *J Hazard Mater* 194:414–421
- Li H, Ye Z, Chan W et al (2011b) Can arbuscular mycorrhizal fungi improve grain yield, As uptake and tolerance of rice grown under aerobic conditions? *Environ Pollut* 159:2537–2545

- Li H, Chen XW, Wong MH (2016) Arbuscular mycorrhizal fungi reduced the ratios of inorganic/organic arsenic in rice grains. *Chemosphere* 145:224–230
- Liu J, Blaylock LA, Endre G et al (2003) Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. *Plant Cell* 15:2106–2123
- Liu Y, Zhu YG, Chen BD et al (2005) Influence of the arbuscular mycorrhizal fungus *Glomus mosseae* on uptake of arsenate by the As hyperaccumulator fern *Pteris vittata* (L.). *Mycorrhiza* 15:187–192
- Lomax C, Liu WJ, Wu LY et al (2012) Methylated arsenic species in plants originate from soil microorganisms. *New Phytol* 193:665–672
- Ma LQ, Komar KM, Tu C et al (2001) A fern that hyperaccumulates arsenic. *Nature* 409:579–579
- Ma JF, Yamaji N, Mitani N et al (2008) Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *Proc Natl Acad Sci U S A* 105:9931–9935
- Mahimairaja S, Bolan NS, Adriano DC et al (2005) Arsenic contamination and its risk management in complex environmental settings. *Adv Agron* 86:1–82
- Maia LC, Trufem SFB (1990) Vesicular-arbuscular mycorrhizal fungi in cultivated soils in Pernambuco State, Brazil. *Rev Bras Bot* 13:89–95
- Mandal BK, Suzuki KT (2002) Arsenic round the world: a review. *Talanta* 58:201–235
- Marin AR, Masscheleyn PH, Patrick WH Jr (1992) The influence of chemical form and concentration of As on rice growth and tissue arsenic concentration. *Plant Soil* 139:175–183
- Matysik J, Alia Bhalu B, Mohanty P (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr Sci* 82:525–532
- Meharg AA (2003) The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. *Mycol Res* 107:1253–1265
- Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytol* 154:29–43
- Meharg AA, Jardine L (2003) Arsenite transport into paddy rice (*Oryza sativa*) roots. *New Phytol* 15:39–44
- Meharg AA, Macnair MR (1992) Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* (L.). *J Exp Bot* 43:519–524
- Meharg AA, Macnair MR (1994) Relationship between plant phosphorous status and the kinetics of arsenate influx in clones of *Deschampsia cepitosa* (L.) Beauv that differ in their tolerance to arsenate. *Plant Soil* 162:99–106
- Meharg AA, Williams PN, Adomako E et al (2009) Geographical variation in total and inorganic arsenic content of polished (white) rice. *Environ Sci Technol* 43:1612–1617
- Mendez M, Maier MR (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. *Environ Health Perspect* 116:278–283
- Meng XG, Korfiatis GP, Bang S et al (2002) Combined effects of anions on arsenic removal by iron hydroxides. *Toxicol Lett* 133:103–111
- Mishra S, Srivastava S, Tripathi RD et al (2006) Phytochelatin synthesis and response of antioxidants during cadmium stress in *Brassica monnieri* (L.). *Plant Physiol Biochem* 44:25–37
- Mishra S, Srivastava S, Tripathi RD et al (2008) Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* (L.). *Aquat Toxicol* 86:205–215
- Miteva E (2002) Accumulation and effect of arsenic on tomatoes. *Commun Soil Sci Plant Anal* 33:1917–1926
- Mukhopadhyay R, Shi J, Rosen BP (2000) Purification and characterization of ACR2p, the *Saccharomyces cerevisiae* arsenate reductase. *J Biol Chem* 275:21149–21157
- Mukhopadhyay R, Rosen BP, Phung LT et al (2002) Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiol Rev* 26:311–325
- Mylona PV, Polidoros AN, Scandalios JG (1998) Modulation of antioxidant responses by arsenic in maize. *Free Radic Biol Med* 25:576–585

- Nagy ML, Johansen JR, Clair St LL et al (2005) Recovery patterns of microbiotic soil crusts, 70 years after arsenic contamination. *J Arid Environ* 63:304–323
- NAS (2000) Arsenic: medical and biological effects of environmental pollutants. Arsenic Natl Acad Sci Washington, DC. doi: <https://doi.org/10.17226/9003>
- Nordstrom DK (2002) Public health-worldwide occurrences of arsenic in ground water. *Science* 296:2143–2145
- Orlowska E, Ryszka P, Jurkiewicz A et al (2005) Effectiveness of arbuscular mycorrhizal fungal (AMF) strains in colonization of plants involved in phytostabilisation of zinc wastes. *Geoderma* 129:92–98
- Patel KS, Shrivastava K, Brandt R et al (2005) Arsenic contamination in water, soil, sediment and rice of central India. *Environ Geochem Health* 27:131–145
- Phillips DJH (1990) Arsenic in aquatic organisms – a review, emphasizing chemical speciation. *Aquat Toxicol* 16:151–186
- Raab A, Williams PN, Meharg A et al (2007) Uptake and translocation of inorganic and methylated arsenic species by plants. *Environ Chem* 4:197–203
- Rahmaty R, Khara J (2008) Effects of vesicular arbuscular mycorrhiza *Glomus intraradices* on photosynthetic pigments, antioxidant enzymes, lipid peroxidation, and chromium accumulation in maize plants treated with chromium. *Turk J Biol* 35:51–58
- Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. *New Phytol* 171:41–53
- Rivera-Becerril F, Van Tuinen D, Martin-Laurent F et al (2005) Molecular changes in *Pisum sativum* (L.) roots during arbuscular mycorrhiza buffering of cadmium stress. *Mycorrhiza* 16:51–60
- Robson AD, Abbott LK (1989) The effect of soil acidity on microbial activity in soils. In: Robson AD (ed) *Soil acidity and plant growth*. Academic Press Australia, New South Wales, pp 139–165
- Roy M, Mukherjee A, Mukherjee S et al (2014) Arsenic: an alarming global concern. *Int J Curr Microbiol App Sci* 3:34–47
- Sairam RK, Srivastava GC, Aggarwal S et al (2005) Differences in antioxidant activity in response to salinity stress intolerant and susceptible wheat genotypes. *Biol Plant* 49:85–91
- Santana AN, Ferreira AAP, Soriani HH et al (2015) Interaction between arbuscular mycorrhizal fungi and vermicompost on copper phytoremediation in a sandy soil. *Appl Soil Ecol* 96:172–182
- Schat H, Sharma SS, Vooijs R (1997) Heavy metal-induced accumulation of free proline in metal tolerant and a nontolerant ecotype of *Silene vulgaris*. *Physiol Plant* 101:477–482
- Schenck NC, Siqueira JO (1987) Ecology of mycorrhizal fungi in temperate agroecosystems. In: Sylvia DM, Hung LL, Graham JH (eds) *7th North American conference on mycorrhizae in the next decade*, Gainesville, pp 2–4
- Schneider J, Laboryb CRG, Rangel WM et al (2013) Anatomy and ultrastructure alterations of *Leucaena leucocephala* (Lam.) inoculated with mycorrhizal fungi in response to arsenic contaminated soil. *J Hazard Mater* 262:1245–1258
- Schnepf A, Roose T, Schweiger P (2008) Impact of growth and uptake patterns of arbuscular mycorrhizal fungi on plant phosphorus uptake: a modelling study. *Plant Soil* 312:85–99
- Schnepf A, Leitner D, Klepsch S et al (2011) Modelling phosphorus dynamics in the soil-plant system. In: Bunemann EK, Obserson A, Frossard E (eds) *Phosphorus in action: biological processes in soil phosphorus cycling*. Springer, Heidelberg, pp 113–133
- Schreiber U, Bilger W, Hormann H et al (1998) Chlorophyll fluorescence as a diagnostic tool: basics and some aspects of practical relevance. In: Ragendra AS (ed) *Photosynthesis: a comprehensive treatise*. Cambridge University Press, Cambridge
- Schulz H, Härtling S, Tanneberg H (2008) The identification and quantification of arsenic induced phytochelatin comparison between plants with varying As sensitivities. *Plant Soil* 303:275–287

- Shaibur MR, Kitajima N, Sugewara R et al (2008) Critical toxicity of arsenic and elemental composition of arsenic-induced chlorosis in hydroponic Sorghum. *Water Air Soil Pollut* 191:279–292
- Sharma I, Singh R, Tripathi BN (2007) Biochemistry of Arsenic toxicity and tolerance in plants. *Biochem Cell Arch* 7:165–170
- Shen J, Hsu CM, Kang BK et al (2003) The *Saccharomyces cerevisiae* Arr4p is involved in metal and heat tolerance. *Biometals* 16:369–378
- Shen H, He Z, Yan H et al (2014) The fronds tonoplast quantitative proteomic analysis in arsenic hyperaccumulator *Pteris vittata* (L.) *J Proteome* 105:46–57
- Sheng M, Tang M, Chen H et al (2008) Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* 18:287–296
- Shin H, Shin HS, Dewbre GR et al (2004) Phosphate transport in Arabidopsis: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. *Plant J* 39:629–642
- Shri M, Smita K, Debasis C (2009) Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedlings. *Ecotoxicol Environ Saf* 72:1102–1110
- Singh KS (2015) Groundwater arsenic contamination in the middle-gangetic plain, Bihar (India): The Danger Arrived. *Int Res J Environ Sci* 4:70–76
- Singh N, Ma LQ, Srivastava M et al (2006) Metabolic adaptations to arsenic-induced oxidative stress in *Pteris vittata* L. and *Pteris ensiformis* L. *Plant Sci* 17:274–282
- Smirnov N, Cumbes QJ (1989) Hydroxyl radical scavenging activity of compatible solute. *Phytochemistry* 28:1057–1060
- Smith SE, Read DJ (eds) (1997) *Mycorrhizal symbiosis*. Academic, San Diego, CA, pp 453–469
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*. Academic, San Diego, CA
- Smith S, Smith F (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annu Rev Plant Biol* 62:227–250
- Smith A, Hopenhayn-Rich C, Bates M et al (1992) Cancer risks from arsenic in drinking water. *Environ Health Perspect* 97:259–267
- Smith SE, Christophersen HM, Pope S et al (2010a) Arsenic uptake and toxicity in plants: integrating mycorrhizal influences. *Plant Soil* 327:1–21
- Smith SE, Facelli E, Pope S et al (2010b) Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil* 326:3–20
- Spagnoletti FN, Lavado SL (2015) The arbuscular mycorrhiza *Rhizophagus intraradices* reduces the negative effects of arsenic on soybean plants. *Agronomy* 5:188–199
- Spagnoletti FN, Balestrasse K, Lavado RS et al (2016) Arbuscular mycorrhiza detoxifying response against arsenic and pathogenic fungus in soybean. *Ecotox Environ Safe* 133:47–56
- Srivastava M, Ma LQ, Singh N et al (2005) Antioxidant responses of hyper-accumulator and sensitive fern species to arsenic. *J Exp Bot* 56:1335–1342
- Stoeva N, Bineva T (2003) Oxidative changes and photosynthesis in oat plants grown in As-contaminated soil. *Bulg J Plant Physiol* 29:87–95
- Stoeva N, Berova M, Zlatev Z (2004) Physiological response of maize to arsenic contamination. *Biol Plant* 47:449–452
- Stoeva N, Berova M, Vassilev A et al (2005) Effect of arsenic on some physiological parameters in bean plants. *Biol Plant* 49:293–296
- Sun Y, Zhang X, Wu Z et al (2016) The molecular diversity of arbuscular mycorrhizal fungi in the arsenic mining impacted sites in Hunan Province of China. *J Environ Sci* 39:110–118
- Takamatsu T, Aoki H, Yoshida T (1982) Determination of arsenate, arsenite, monomethylarsonate, and dimethylarsinate in soil polluted with arsenic. *Soil Sci* 133:239–246
- Tchabi A, Coyne D, Hountondji F et al (2008) Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. *Mycorrhiza* 18:181–195

- Tiwari M, Sharma D, Dwivedi S et al (2014) Expression in Arabidopsis and cellular localization reveal involvement of rice NRAMP, OsNRAMP1, in arsenic transport and tolerance. *Plant Cell Environ* 37:140–152
- Trotta A, Falaschi P, Cornara L et al (2006) Arbuscular mycorrhiza increases the arsenic translocation factor in the arsenic hyperaccumulating fern *Pteris vittata*. *Chemosphere* 65:74–81
- Tseng YY, Yu CW, Liao VHC (2007) *Caenorhabditis elegans* expresses a functional ArsA. *FEBS J* 274:2566–2572
- Tu S, Ma LQ (2003) Effects of arsenate and phosphate on their accumulation by an arsenic hyperaccumulator *Pteris vittata* (L.) *Plant Soil* 249:373–382
- Turnau K, Kottke I, Obserwinkler F (1993) Element localization in mycorrhizal roots of *Pteridium aquilinum* (L.) Kuhn collected from experimental plots treated with cadmium dust. *New Phytol* 123:313–324
- Ultra V, Tanaka S, Sakurai K et al (2007) Effects of arbuscular mycorrhizae and phosphorous application on arsenic toxicity in sunflower (*Helianthus annuus* L.) and on the transformation of arsenic in the rhizosphere. *Plant Soil* 290:29–41
- Vivas A, Vörös I, Biro B (2003) Symbiotic efficiency of autochthonous arbuscular mycorrhizal fungus (*G. mosseae*) and *Brevi bacillus* sp. isolated from cadmium polluted soils under increasing cadmium levels. *Environ Pollut* 126:179–189
- Wang JR, Zhou FJ, Meharg AA et al (2002) Mechanisms of arsenic hyperaccumulation in *Pteris vittata* uptake kinetics, interactions with phosphate, and arsenic speciation. *Plant Physiol* 130:1552–1561
- Wang FY, Lin XG, Yin R (2007) Role of microbial inoculation and chitosan in phytoextraction of Cu, Zn, Pb and Cd by *Elsholtzia splendens* – a field case. *Environ Pollut* 147:248–255
- Weissenhorn I, Leyval C, Belgy G et al (1995) Arbuscular mycorrhizal contribution to heavy-metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. *Mycorrhiza* 5:245–251
- Whitfield L, Richards AJ, Rimmer DL (2004) Relationships between soil heavy metal concentration and mycorrhizal colonisation in *Thymus polytrichus* in northern England. *Mycorrhiza* 14:55–62
- Wright SF, Upadhyaya A (1996) Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci* 161:575–586
- Wu FY, Ye ZH, Wu SC et al (2007) Metal accumulation and arbuscular mycorrhizal status in metallicolous and non-metallicolous populations of *Pteris vittata* L. and *Sedum alfredii* Hance. *Planta* 226:1363–1378
- Wu QS, Zou YN, Liu W et al (2010) Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: changes in leaf antioxidant defense systems. *Plant Soil Environ* 56:470–475
- Wu Z, Ren H, McGrath SP et al (2011a) Investigating the contribution of the phosphate transport pathway to arsenic accumulation in rice. *Plant Physiol* 157:498–508
- Wu J, Van Geen A, Ahmed KM et al (2011b) Increase in diarrheal disease associated with arsenic mitigation in Bangladesh. *PLoS One* 6:29593. <http://dx.doi.org/10.1371/journal.pone.0029593>
- Xia YS, Chen BD, Christie P et al (2007) Arsenic uptake by arbuscular mycorrhizal maize (*Zea mays* L.) grown in an arsenic contaminated soil with added phosphorus. *J Environ Sci* 19:1245–1251
- Xiang D, Verbruggen E, Hu Y et al (2014) Land use influences arbuscular mycorrhizal fungal communities in the farming-pastoral ecotone of northern China. *New Phytol* 204:968–978
- Xu T, Cai Y, O'Shea KE (2007) Adsorption and photocatalyzed oxidation of methylated arsenic species in TiO₂ suspensions. *Environ Sci Technol* 41:5471–5477
- Xu PL, Christie P, Liu Y et al (2008) The arbuscular mycorrhizal fungus *Glomus mosseae* can enhance arsenic tolerance in *Medicago truncatula* by increasing plant phosphorus status and restricting arsenate uptake. *Environ Pollut* 15:215–220
- Yang J, Ajees AA, Salam A et al (2011) Genetic mapping of the interface between the ArsD metallochaperone and the ArsAATPase. *Mol Microbiol* 79:872–881

- Ye J, Ajees AA, Yang J et al (2010) The 1.4 Å crystal structure of the ArsD ar metallochaperone provides insights in to its interaction with the ArsA ATPase. *Biochemistry* 49:5206–5212
- Yeh S, How SW, Lin CS (1968) Arsenical cancer of skin-histologic study with special reference to Bowen's disease. *Cancer* 21:312–339
- Zhang SY, Rensing C, Zhu YG (2014) Cyanobacteria-mediated arsenic redox dynamics is regulated by phosphate in aquatic environments. *Environ Sci Technol* 48:994–1000
- Zhang X, Ren BH, Wu SL et al (2015) Arbuscular mycorrhizal symbiosis influences arsenic accumulation and speciation in *Medicago truncatula* L. in arsenic contaminated soil. *Chemosphere* 119:224–230
- Zhao FJ, Wang JR, Barker JHA et al (2003) The role of phytochelatin in arsenic tolerance in the hyperaccumulator *Pteris vittata*. *New Phytol* 159:403–410
- Zhao FJ, Ma JF, Meharg AA et al (2009) Arsenic uptake and metabolism in plants. *New Phytol* 181:777–794
- Zhao FJ, McGrath SP, Meharg AA (2010) Arsenic as a food-chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annu Rev Plant Biol* 61:535–559
- Zhou S, Sauvé R, Thannhauser TW (2010) Proteome changes induced by aluminium stress in tomato roots. *J Exp Bot* 60:1849–1857
- Zhu YG, Rosen BP (2009) Perspectives for genetic engineering for the phytoremediation of arsenic contaminated environments: from imagination to reality? *Curr Opin Biotechnol* 20:220–224
- Zhu YG, Smith FA, Smith SE (2003) Phosphorus efficiencies and responses of barley (*Hordeum vulgare* L.) to arbuscular mycorrhizal fungi grown in highly calcareous soil. *Mycorrhiza* 13:93–100

Chapter 8

Co-cultivation of *Piriformospora indica* with *Azotobacter* sp.

Prasun Bandyopadhyay and Ajit Varma

Abstract The ever growing human population and depletion of resources have enforced progress in sustainable agriculture. Plant–rhizosphere microbe association has been known for some time now. To uphold sustainability, one would need to show better reliance upon the beneficial traits possessed by the root microbiome. To harness these traits, one would need to understand the process of recruitment and maintenance of microbiota as stable interactome. In this chapter, we highlight the process of recruitment and establishment of microbiota within rhizosphere. Further, we discuss the molecular basis of interspecies synergistic interaction where we have taken *Piriformospora indica* and *Azotobacter chroococcum* as model interacting partners. Lastly, we laid emphasis on the possibility of exploring the knowledge gained from such synergistic interaction to tailor the rhizosphere microbiome for better productivity and maintenance of agroecosystem. This chapter provides new insights into the broad principles of stable plant–microbe interactions which could be useful for sustaining agriculture and food security.

8.1 Introduction

Since the time humans have emerged, population has increased at a slow and steady pace. However, the past 100 years have witnessed tremendous increase than ever before, and it is expected to reach 9.6 billion by the end of 2050. If the population keeps on increasing in such an alarming rate, it may challenge the food security. As majority of the population depends on the agricultural sector, improving the quality and productivity will be advantageous. Therefore, considering the importance of the agricultural sector, it has become vital to ensure that genetic diversity is maintained for higher productivity and further avoidance of vulnerability to abiotic and biotic stress. Widespread research in plant–microbe association has highlighted the importance of microorganisms in determining the plant fitness (Gill et al. 2016).

P. Bandyopadhyay • A. Varma (✉)
Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, Noida 201303,
India
e-mail: ajitvarma@amity.edu

Thus, understanding the genesis of plant–microbe beneficial interaction will be advantageous in context to improve productivity.

Plants are usually found to be densely inhabited by diverse range of microorganisms both above and below the ground drawing mutual benefits. Depending on the niches of the plant colonized by microorganisms, they may be designated as epiphyte (present on the surface), endophyte (found inside the tissue), phyllospheric (growing on leaf surface), and rhizospheric (inhabiting soil closely associated with roots). Out of these diverse niches, rhizosphere is the most dynamic owing to its massive influence on plant nutrition and growth (Kowalchuk et al. 2010; Lakshmanan et al. 2014, Berendsen et al. 2012; Bakker et al. 2013; Mendes et al. 2013; Prasad et al. 2015). Given the enormous species diversity, staggering number of interactions, and complex community structure within the rhizosphere, understanding the biology of the root system and its microbiota as an interactome is still at its infancy. Plant hosts and their microbiota are often intertwined and are thought to have coevolved and function as meta-organism or holobiont having inseparable ecology (Bosch and McFall-Ngai 2011; Vandenkoornhuysen et al. 2015). To appreciate such a holobiont system, one needs to understand the interdependence of microbiota at various strata of their growth and development and may even view a plant's biology as the additive functions of the surrounding microbiota. With the advent of genomics and proteomics, recent scientific literature has explored the mechanisms coordinating the formation of plant–microbe mutualistic associations having the potential to improve plant productivity (Mabood et al. 2008; Pieterse et al. 2009; Berendsen et al. 2012; Bakker et al. 2013). However, the basis of microbe–microbe interaction is still at its early stage. So, if the factors that contribute to the formation of stable rhizosphere community are deciphered, they can be harnessed for sustainable agriculture to address the ever-increasing demand of food.

This chapter provides an outline of the root microbiome dynamics with an emphasis on the recruitment of the microbiota in the rhizosphere, considering bulk soil as a microbial sink. Further, we also address the formation of stable interspecies interactomes. Here, we have discussed upon molecular basis of trophic interaction between *Piriformospora indica* and *Azotobacter chroococcum*. Finally, we laid emphasis on the possibility of exploring the knowledge gained from such trophic interaction to tailor the rhizosphere microbiome for better productivity and maintenance of agroecosystem.

8.2 Rhizosphere as Microbial Incubator: Recruiting Microorganisms to Root Niche

Recruitment of root microbiota has gained much attention as it influences plant health and productivity. Ever since the origin of first terrestrial plants, a new set of biological factors have been introduced for the soil microorganism to deal along

with (Selosse and Strullu-Derrien 2015) the physiochemical factors. To understand the genesis behind the recruitment of microorganisms in the rhizosphere, one has to decipher their species and functional diversity. Though considerable progress has been made, it remains a huge challenge as currently only 1% of the entire soil dwelling microorganisms are cultivable (Walsh and Duffy 2013). With the development of omics and culture-independent techniques, scientists have been able to gain insight into rhizosphere microbiota. High throughput techniques have yielded estimates of up to 10^{11} microbial cells per gram of root which include at least 30,000 prokaryotes. These estimates may vary depending upon the plant species, genotype, and age. We begin this section by summarizing the role of root exudates as potential drivers, attracting microorganisms to the desired rhizosphere niche from the bulk soil.

Lorenz Hiltner coined the term “rhizosphere” to describe influence of root exudates on the soil microorganisms proliferating around and inside the roots (Hirsch and Mauchline 2012). Ever since rhizosphere has been defined, much has been learned on the effect of soil microorganisms on the plant host. In natural ecosystem, plants have been the driving force for assembling the rhizosphere microbiota constituting the diverse functional gene pool, including bacteria, fungi, and nematodes associated with various habitats like rhizosphere, rhizoplane, and endosphere. Plants tend to release 10–20% of their photosynthates as exudates including low molecular weight metabolites like sugar, organic acids, amino acids, and the dead border cells as mucilage (Dennis et al. 2010; Kaiser et al. 2015). These exudates alter the chemical and physical properties of the soil and cater the niche for microbial proliferation (Bais et al. 2006). Studies conducted on potato, sugarcane, and certain model plants like *Arabidopsis* and Barley have suggested genotype-dependent variation in the microbial community within rhizosphere. Hence, higher microbial count is obtained in rhizosphere in comparison to the bulk soil (Bulgarelli et al. 2012, 2015). These findings suggest a plant system can orchestrate its rhizobiota to optimize its own benefits and improve its fitness. Depending on the type of exudates, they may attract or repel certain groups of microbiota. Badri et al. (2013) demonstrated that exudates, particularly phenolics, can either stimulate or repel certain group of microorganisms highlighting the potential of the exudates in modulation of the microbial communities in the rhizosphere.

The association of microbes with plants may vary from obligate (endo) symbionts to transitory associates. Though ancestrally, prokaryotes and lower eukaryotes persisted as free-living microorganisms, the past millions of years have witnessed a transformation in their ability to socialize and create a niche where they can coordinate interactions. The diverse microbe–microbe and plant–microbe associations within the rhizosphere have received growing attention with regard to abundance, diversity, and complexity and hence considered as plant’s secondary genome. They are often witnessed having major impacts on plant growth (Broeckling et al. 2008; Bulgarelli et al. 2012).

8.3 Formation of Stable Microbial Interaction May Influence Plant Growth and Fitness

Stable cooperation among microbial partners are comparatively unexplored as their mutual responses are difficult to observe. Once functionally diverse microorganisms have been assembled in the rhizosphere, it is important to identify the factors involved in the formation of stable yet dynamic communities. Keeping this in mind, here we have taken *Piriformospora indica* and *Azotobacter chroococcum* as model systems to study the molecular basis of stable interaction.

The mutualistic associations established by mycorrhizal fungi with plant and bacterial cells can range from seemingly disordered polymicrobial communities to highly specific symbiotic associations. Such association has been vastly seen in agricultural as well as forest ecosystems. Many bacteria and fungi either in combination or in isolation have been shown to produce beneficial effects on plants. Interaction between *Pseudomonas putida* and *Glomus* sp. has been shown to promote plant growth by enhancing phosphate solubilization (Villegas and Fortin 2002, 2011). Certain signaling metabolites of *Streptomyces* sp. Ach505 have also been shown to stimulate the hyphal growth of *Amanita muscaria*. Similarly, volatile substances produced by some bark beetle-associated bacteria stimulate the growth of their symbiotic fungi. Few PGPRs like *P. putida* IsoF promoted the growth of *P. indica* whereas *Pseudomonas fluorescens* WS5 and *Gluconacetobacter* sp. Comb19 inhibited fungal growth (Varma et al. 2012). Though this kind of interactions is known to occur in the rhizospheric region, the exact nature of molecular interaction is yet to be elucidated.

To address this, we conducted interaction between *P. indica* and strains of *Azotobacter chroococcum* in axenic culture. Initial in vitro screening revealed different patterns of the growth modulating interactions of strains of *A. chroococcum* with *P. indica*. We identified two strains—WR5 and M4 which have the tendency to modulate the fungal growth. WR5 has maximal growth promoting effect, and M4 strain has maximal growth inhibiting effect as seen in plate assay, dry cell weight content, and spore yield (Fig. 8.1). Electron microscopic (SEM and TEM) observation of *P. indica* did elucidate marked differences in the surface morphology and internal compartmentalization of cytoplasm and membranous organelles in interaction with WR5 and M4. Presence of healthy, thick hyphae in interaction indicates that WR5 supports fungal growth. Contrasting observations have been made in the presence of M4, where the hyphal architecture has been highly deformed. The cytoplasm has been disorganized in comparison to control. This suggests that in presence of M4, the fungus is metabolically less active (Figs. 8.2 and 8.3).

Further, to explore the mechanism of growth modulation of *P. indica* by both the strains, we demonstrated that WR5 and M4 lead to a specific modulation of protein expression in *P. indica*. In particular, *P. indica* cocultured with WR5 showed an increase in the level of expression of some major metabolic proteins. The latter were downregulated in the presence of M4. Based on a comparative analysis of

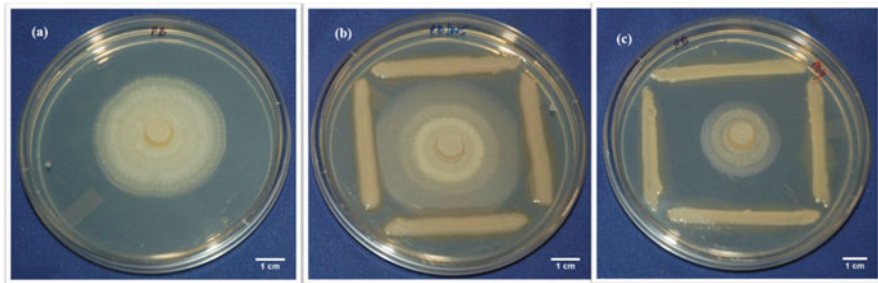


Fig. 8.1 Visualization of the *A. chroococcum*—*P. indica* interaction in Hill and Kaefer agar plates in the presence and absence of *A. chroococcum* strains. (a) Control plate. (b) Interaction of *P. indica* with WR5. (c) Interaction of *P. indica* with M4

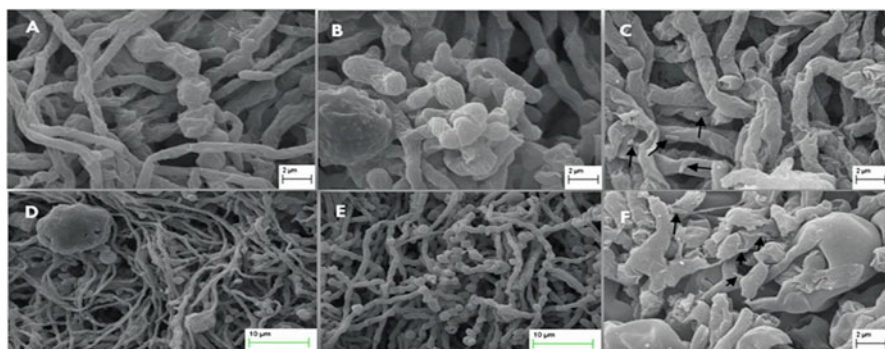


Fig. 8.2 Scanning electron micrographs of *P. indica* morphology in isolation and coculture with *A. chroococcum* (WR5 and M4) specific elicited fungal growth at $28 \pm 1^\circ\text{C}$. (A and D) Control fungal mycelia appear to have normal hyphae, septa, and conidia. Hyphae showed uniform tubular shape in all parts. (B and E) Micrographs show a tendency of hyphal growth promotion induced by WR5. The main improvements are healthy fungal hyphae and more conidiation. (C and F) Hyphal growth affected by M4 showing damaged fungal hyphae with surface adhered rod-shaped bacteria (arrow in Fig. 8.2c) and lack of conidiation

major differentially expressed proteins, we present a hypothetical model in Fig. 8.4 suggesting the possible role of WR5 in stimulating the growth of *P. indica*. Upregulation of both ENO1 and Ure D in the presence of WR5 suggests that WR5 could trigger efficient uptake of hexose sugar by the activation of several glucose transporters. ENO1 is one of the key regulatory enzymes of glycolytic pathway for generating reducing power for ATP synthesis. Ure D is one of the accessory proteins of the apoprotein UreABC which is a nickel-dependent regulatory enzyme involved in recycling of urea. Ammonia generated by urease reaction is used as a source of nitrogen by the plant for its growth. This carbon supply generates the metabolic energy *via* glucose metabolism thus prompting the growth of *P. indica*. This has been well supported by increase in number of mitochondria as seen in cocultures with WR5. As the model reflects, the energy generated and the

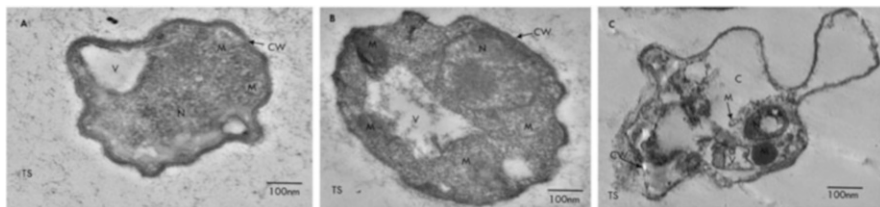


Fig. 8.3 Transmission electron micrographs of *P. indica* in isolation and in coculture with *A. chroococcum* (WR5 and M4) grown at $28 \pm 1^\circ\text{C}$. (A) Transverse section of control hypha showing cell wall (CW) mitochondria (M), vesicles (V), and nucleus (N). (B) Transverse section of hypha from coculture with WR5 well-organized hyphal cytoplasm, organelles, and number of mitochondria. (C) Transverse section of hypha treated with M4 showing disorganization of hypha and cytoplasmic organelles and formation of membrane-bound vesicles

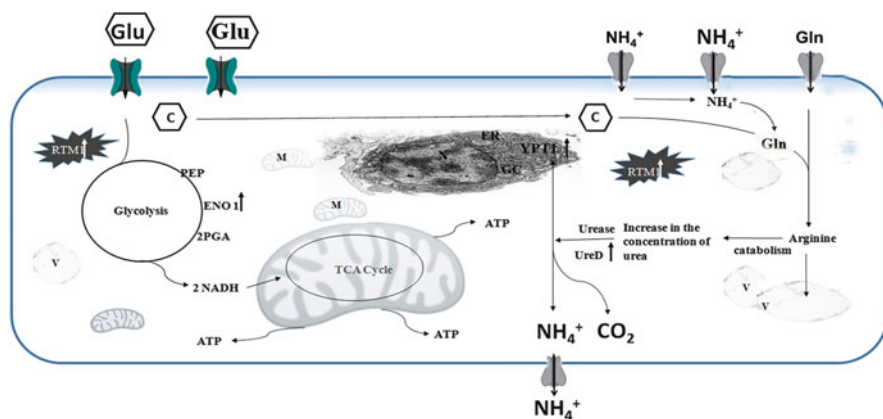


Fig. 8.4 A hypothetical model showing changes in the carbon and nitrogen flux of *P. indica* in response to its interaction with WR5. Presence of WR5 enhances carbon pool in the mycelium triggering inorganic/organic nitrogen influx. This nitrogen is assimilated via glutamine synthetase/glutamate synthase cycle into arginine. Arginine is fed in the urea cycle; urea is further broken down to ammonia and carbon dioxide which is released. Stimulation in the expression of ENO1 and Ure D during carbon and in-/organic nitrogen influx has been indicated by up-arrows

carbon source may be further utilized in the active uptake processes of inorganic and organic nitrogen source by the mycelium further assimilating it into amino acid—Arginine, which is loaded into the vacuoles and transported along the hypha. As a regulatory process, arginine is loaded into the anabolic arm of the urea cycle in order to be degraded, leading to an increased concentration of urea. In the presence of active urease, urea can be converted into ammonia and carbon dioxide.

In conjunction with the activity of ENO1 and Ure D, YPT-1 and RTM1 proteins have unique importance in the growth of the fungus. YPT-1 does play a functional role in sporulation and the organization of the cytoskeleton during the vegetative state as it has been well documented in *S. cerevisiae*. In addition, it has also been identified as global GTP-binding protein associated with trafficking of secretory



Fig. 8.5 Chlamydospore germination pattern of *P. indica* after 12 h growth in Hill and Kaefler minimal medium in the absence (a) and presence of WR5 (b) and M4 (c) cell-free supernatant. Chlamydospores, grown in axenic culture, are at the initial stage of germ tube formation, whereas in the presence of WR5 cell-free supernatant, clear and distinct germinating tube is observed. In the presence M4 cell-free supernatant, growth is suppressed prior to germination. Images were taken at 400-fold magnification

vesicles between endoplasmic reticulum and Golgi complex. *RTM1* is a membrane-bound protein known to provide immunity and resistance to the fungus from the environmental toxic compounds. Similar pattern of response was observed in interaction of *L. bicolor* with soil bacteria though different sets of genes/proteins were found to be involved in the interaction (Deveau et al. 2015).

The specific growth response of the fungus could be caused by specific bacterial metabolites released into the environment during co-cultivation. To test whether diffusible low molecular weight active signaling molecules elicit the fungal growth response, we performed fungus growth assessment with 20-fold concentrates of culture supernatants. The results obtained from these experiments suggest that the cell-free culture supernatant might contain active metabolites for specific growth response of the fungus. The metabolites produced by WR5 contributed to early and better spore germination of *P. indica* with much elongated germ tube rising from the spores whereas in presence of M4 the spores did not show any visible mark of spore germination. In fact the spores were round suggesting that metabolites released from M4 suppressed the spore germination of *P. indica* and hence the overall growth (Fig. 8.5) (Bandyopadhyay et al. 2016a). This is in good agreement with our previous study, where WR5 was shown to improve fungal growth in terms of biomass and sporulation (Bhuyan et al. 2015). This also matches with a study conducted by Lumini et al. (2007), where endobacteria harbored by the arbuscular mycorrhizal fungus *Gigaspora margarita* were found to play a pivotal role in spore germination during the pre-symbiotic stage of infection. This observation reflects the potential strategy employed by free-living bacteria to attract mutualistic fungi and to repel competing bacteria from the same niche by manipulating their physiology with secreted factors.

8.4 Secreted Factors Involved in *P. indica*–*A. chroococum* Perception

For the identification of secretory factors involved in mutual perception, fungi and bacteria were cocultured, and proteins in the supernatant were extracted and analyzed by SDS-gel electrophoresis. Gel analysis of secreted proteins resolved numerous bands, ten protein bands of which were identified as differentially expressed. They are annotated as band A to J with molecular masses of approximately 37 kDa (A), 37 kDa (B), 40 kDa (C), 40 kDa (D), 73 kDa (E), 70 kDa (F), 73 kDa (G), 70 kDa (H), 73 kDa (I), and 70 kDa (J) (Figs. 8.2 and 8.3). Two distinct bands, A and B, were found to be upregulated by WR5 supernatant alone as well as in coculture with *P. indica*. Interestingly, two bands (E and F) of control *P. indica* cultures were found to be downregulated in coculture with WR5 (I and J). A similar result was obtained with M4 supernatants for bands G and H. Bands C and D (40 kDa) show ample expression with both M4-fungal coculture and M4 alone (Fig. 8.6).

To identify and elucidate the role of these secreted proteins involved in the *P. indica* growth modulation, they were subjected to LC-ESI-MS/MS analysis. For identification, proteins having individual ion score values >100 were selected. Band A of WR5 was found to be a flagellar domain protein. Band B was identified as glutamate dehydrogenase belonging to *P. indica*. Bands C and D were identified as probable flagellar biosynthesis protein FliC from *A. chroococum* M4 with a score of 368 and the mass 40.05 kDa and flagellin with a score of 314 and the mass 40.42 kDa. Proteins E and F correspond to α -glucosidase-b like secreted protein of *P. indica* with a score of 163 and the mass 98.83 kDa.

WR5 was shown to stimulate fungal growth, similar to observations reported earlier (Bhuyan et al. 2015). In this communication, we propose that the flagellar domain protein of WR5 induces the expression of fungal glutamate dehydrogenase. Glutamate dehydrogenase is usually involved in assimilation of inorganic nitrogen,

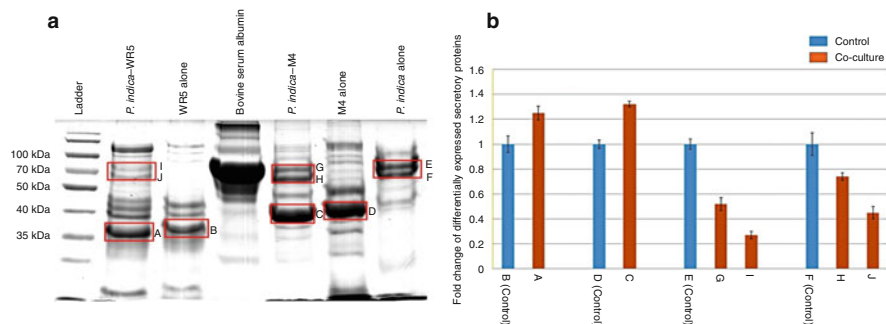


Fig. 8.6 SDS-PAGE analysis of secreted proteins of axenically grown *P. indica* or in coculture with WR5 and M4 (a). Changes in differentially expressed secretory proteins of *P. indica* and *A. chroococum* grown axenically or in coculture (b). Data were obtained by integrated densitometry values of the bands analyzed. Notations correspond to those in a

synthesizing glutamate from ammonium and α -ketoglutarate. Being a reversible enzyme, it can also degrade glutamate, yielding ammonium and α -ketoglutarate depending upon the concentration of ammonium. However, ammonium ions are toxic to the cell. Glutamate dehydrogenase converts it into glutamate, feeding the urea cycle for assimilating ammonium ion in the organic form (Bandyopadhyay et al. 2016a). This matches with our previous study, where glucose was shown to trigger nitrogen uptake in *P. indica* in the presence of WR5. Remarkably, α -glucosidase b of *P. indica* was found to be downregulated in the presence of WR5. α -Glucosidase b is a cell wall degrading enzyme, possessed by saprophytes and involved in invading the host by the pathogen (Favre et al. 2014). Downregulation of this protein suggests that WR5 enhances the beneficial relationship between *P. indica* and the plant by minimizing saprophytic traits of *P. indica*.

In interaction with M4, no such demarking proteins were identified that could elucidate the role of M4 in inhibiting fungal growth. However, the flagellar biosynthesis protein FliC was found to be highly expressed in both, M4 alone and in coculture with *P. indica*. An earlier report with *Pseudomonas aeruginosa* suggested that FliC could be involved as Type III secretion protein antagonizing the host by its toxicity. It may be assumed that the FliC protein secreted by M4 could play a similar role in antagonizing fungal growth. However, this study needs further data mining and additional validation to identify factors involved in inhibition of fungal growth. The interaction between the bacterium and the fungus may also involve secretion of volatiles and small diffusible secondary metabolites. Saponins may be seen as a reasonable assumption in this respect. Results obtained with observations on growth and motility of the bacteria *Collimonas pratensis* Ter291 and *Serratia plymuthica* Pri-2C in response to terpenes, secreted by *Fusarium culmorum*, point into a comparable direction. Several studies showed that fungi react with high sensitivity to volatiles emitted by bacteria, leading to reduction and inhibition of spore germination and growth (Schmidt et al. 2016). Similarly, *Streptomyces* AcH 505 has been shown to stimulate ectomycorrhizal growth by secreting auxofuran, whereas AcM11 inhibited it by secreting cycloheximide. This is also in close agreement with the studies by Bhuyan et al. (2015), where concentrated preparations of the cell-free *A. chroococcum* supernatant were shown to contain active fractions influencing fungal growth. This suggests that bacterial–fungal interactions may not simply depend on a single factor but instead on a blend of modulators, working together in modulating the phenotypic response that depends in addition on growth stage and nutrient conditions of the interacting partners.

The interaction of microorganisms with plant rhizosphere is not an autecological issue and has serious secondary impact on the performance of the plant vis-à-vis other organisms in its surroundings. Repeated selection for single traits in cultivars must have narrowed their interaction with microbiota in the rhizosphere. It has been argued that studies defining factors that determine the nature of microbiome would have to integrate results of systems-based approach in rhizospheric soil ecosystem. Plant species, soil type, agricultural practices, climatic factors, plant community structure, and nature of biotic interaction of microbes with plants would together

determine the course of plant–microbe co-evolution. The below-ground biological interactions influence biochemical constitution and behavior of aboveground multitrophic organisms including pests and pathogens. This illustrates the probable relevance of the present observations on differentially expressed proteins of *P. indica* and *Azotobacter* for developing cultivars for sustainable agriculture by rhizosphere engineering.

8.5 Rhizosphere Microbiome Engineering: Novel Approach to Improve Plant Fitness and Restore Agro-Ecosystem

Deterioration of the biosphere and the global issue related to growing human population has raised the importance of alternate strategy to improve soil fertility and plant productivity. Global community has been intensively depending on the external inputs of chemical fertilizers to enhance production. However, such practices may deplete natural resources and weaken the ability of the agro-ecosystems to sustain production in the future. Development of transgenic plants could improve the fitness of the plant but has not been gaining importance because of the global ethical issues. Though many such approaches have been devised, engineering the microbial community has been found to be promising and recently gained importance. Microbiome engineering approach incorporates microbiota having ecologically distinct functions into niche to improve ecological balance and reduce anthropogenic inputs (Bandyopadhyay et al. 2016b). Thus, inputs of beneficial microorganism have been promising. These features make the *P. indica*–*A. chroococcum* co-inoculation of non-legume crops most promising for optimal plant production. Along with *P. indica*, strains like WR5 can be a better choice as nitrogen biofertilizer in cold region or the region like Thar Desert in India where plants experience a variety of stresses like drought, heat (summer), and cold (winter). A field trial of *A. chroococcum* strain M4 inoculation with maize demonstrated a significant increase in yields and saving of nitrogen fertilizer when applied in combination with farmyard manure. The M4 strain can be further explored for its application as biocontrol for fungal pathogens associated with crops along with biofertilizer. The growth-promoting and inhibiting effects of *A. chroococcum* strains and their secondary metabolites on beneficial fungi have enormous potential for agronomical applications. Identification and validation of the potent molecular modulators (secondary metabolites) present in the culture supernatants and studying its effect on cellular genes of the fungus by undertaking a genome-wide profiling of transcripts of *P. indica* will help establish the mechanism of such interactions.

However, it raises question if such beneficial relationship can be maintained and sustained even in disease prone zones. The existence of beneficial plant–microbe relationship in disease suppressive soil encourages external inputs of the beneficial

microbiota to enhance productivity. Plants grown in such soil are either less challenged or are free of pathogens. The microorganisms can be incorporated as biofertilizers or may be as potential inoculants tailoring the native microbiome. Of late root endophytic fungus *Piriformospora indica* has gained importance in the quest of agronomical functional traits for sustainable agriculture. *P. indica* has been found to mimic AMF with respect to its ability to enhance phosphate uptake and protect the host plant from abiotic and biotic stress (Lahrmann et al. 2013). This raises the importance of adding beneficial microbiota as inoculants in maintaining the ecological functions and sustaining agriculture for improved food security. Some plants possess the ability to restrict nitrification process (transformation of ammonium to nitrate) by affecting nitrifying microorganisms. Thus increasing the available nitrogen and preventing leaching (Bender et al. 2016). Recent study done on interaction of *Azotobacter* with *Brassica napus* demonstrated enhanced root branching, plant biomass, seed yield, and oil content posttreatment (Namvar and Khandan 2015). Secretion of hormones like indole acetic acid, chelators, and various important metabolites like amino acids could play an important role in enhancing plant growth and productivity (Vacheron et al. 2013). These findings have provided valuable model system that could enforce microbiome approach to improve agricultural productivity.

Considerable effort has been made to understand the ecological significance of such bacterial fungal interactions in the rhizosphere. It has been found that even certain environmental changes could trigger free-living organisms to be mutualistic without necessitating adaptive co-evolution. This is in good agreement with the experimental evidences for mutualistic association between free-living organisms being induced by environmental conditions. However, the degree of progression in the lines of mutualism seems to be depending on species-specific traits. To address this issue, further environmental studies supported by biochemical and molecular profile of such interactions can be undertaken.

8.6 Conclusion and Future Remarks

In this chapter, we have tried to develop an understanding on formation of stable microbial assemblage in the rhizosphere system and its influence on plant fitness and productivity. Significant progress made in this area of research has opened up many unsolved puzzles. At community level, the plant system behaves as a holobiont and is never in isolation. Though genomes of the individual organisms are being sequenced, still it may not provide the entire information about the existence of plant within the ecosystem. Hence, there is need of holistic approach for integrative studies on plant–microbial community. With the development of molecular tools, one may even find the genes that are involved in acquiring reciprocal benefits from the plant–microbial community and harness them to sustain the agriculture. By implementing stable isotope and metagenomics approaches, one will also be able to understand the underlying food web connecting

the microbial communities of different rhizosphere niche. Increase in the population has been demanding new crops and implementation of alternate cropping system. With the application of beneficial microbial partners, methods need to be strategized for adequate plant productivity at lower environmental costs. In the recent past, advances made in understanding root microbiome have provided important cues related to formation and maintenance of the root microbial assembly. The next important step is to mine the beneficial plant attributes that are programmed by the rhizospheric microbial assembly. Based on this approach, microbiome can be manipulated/ engineered to test their efficacy towards host plant fitness. For example, artificial interaction of gnotobiotic hosts with certain microbial assemblies may give us an idea to elucidate the underlying roles of microbiomes in influencing host performance. Thus, from these insights it is clear that emergence of rhizosphere microbiota as stable interactome holds a great promise for the production of sustainable crops and open up new avenues for directed ecological intensification.

Acknowledgement Authors are thankful to Department of Biotechnology for providing partial funding and Department of Science and Technology for providing Confocal Facility.

References

- Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *J Biol Chem* 288:4502–4512
- Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* 57:233–266
- Bakker PAHM, Berendsen RL, Doornbos RF, Wntermans PCA, Pieterse CMJ (2013) The rhizosphere revisited: root microbiomics. *Front Plant Sci* 4:165. doi:10.3389/fpls.2013.00165
- Bandyopadhyay P, Bhuyan S, Yadava PK, Varma A (2016a) Soluble factors from *Azotobacter chroococcum* modulate growth of *Piriformospora indica* in co-cultures. *Endocytobiosis Cell Res* 27:9–13
- Bandyopadhyay P, Bhuyan SK, Yadava PK, Varma A, Tuteja N (2016b) Emergence of plant and rhizospheric microbiota as stable interactomes. *Protoplasma*. doi:10.1007/s00709-016-1003-x
- Bender FS, Wagg C, van der Heijden GA (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends Ecol Evol* 31(6):440–452. doi:10.1016/j.tree.2016.02.016
- Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci* 17:478–486
- Bhuyan SK, Bandyopadhyay P, Kumar P, Mishra DK, Prasad R et al (2015) Interaction of *Piriformospora indica* with *Azotobacter chroococcum*. *Sci Rep* 5:13911. doi:10.1038/srep13911
- Bosch TCG, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. *Zoology* 114:185–190
- Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. *Appl Environ Microbiol* 74:738–744
- Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E et al (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* 488:91–95

- Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, McHardy AC, Schulze-Lefert P (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* 17:392–403
- Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiol Ecol* 72:313–327
- Deveau A et al (2015) Pairwise transcriptomic analysis of the interactions between the ectomycorrhizal fungus *Laccaria bicolor* S238N and three beneficial, neutral and antagonistic soil bacteria. *Microb Ecol* 69(1):146–159. doi:10.1007/s00248-014-0445-y
- Favre P, Bapaume L, Bossolini E, Delorenzi M, Falquet L, Reinhardt D (2014) A novel bioinformatics pipeline to discover genes related to arbuscular mycorrhizal symbiosis based on their evolutionary conservation pattern among higher plants. *BMC Plant Biol* 14:333. doi:10.1186/s12870-014-0333-0
- Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. *Front Microbiol* 7:332. doi:10.3389/fmicb.2016.00332
- Hirsch PR, Mauchline TH (2012) Who's who in the plant root microbiome? *Nat Biotechnol* 30:961–962
- Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M et al (2015) Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. *New Phytol* 205:1537–1551
- Kowalchuk GA, Yergeau E, Leveau JHJ, Sessitch A, Bailey M (2010) Plant-associated microbial communities. In: Liu W-T, Jansson JK (eds) *Environmental molecular microbiology*. Caister Academic Press, Poole
- Lahrman U, Ding Y, Banhara A et al (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. *Proc Natl Acad Sci U S A* 110:13965–13970
- Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an aboveground problem. *Plant Physiol* 166:689–700
- Lumini E, Bianciotto V, Jargeat P, Novero M, Salvioli A, Faccio A, Bécard G, Bonfante P (2007) Presymbiotic growth and spore morphology are affected in the arbuscular mycorrhizal fungus *Gigaspora margarita* cured of its endobacteria. *Cell Microbiol* 9:1716–1729
- Mabood F, Jung WJ, Smith DL (2008) Signals in the underground: microbial signaling and Plant productivity. In: Nautiyal CS, Dion P (eds) *Molecular mechanisms of plant and microbe coexistence*. Springer, Berlin, pp 291–318
- Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol Rev* 37:634–663
- Namvar A, Khandan T (2015) Inoculation of rapeseed under different rates of inorganic nitrogen and sulfur fertilizer: impact on water relations, cell membrane stability, chlorophyll content and yield. *Arch Agron Soil Sci* 61:1137–1149
- Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. *Nat Chem Biol* 5:308–316
- Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) *Plant growth-promoting rhizobacteria and medicinal plants*. Springer, Switzerland, pp 247–260
- Schmidt R, Etalo DW, de Jager V, Gerards S, Zweers H, de Boer W, Garbeva P (2016) Microbial small talk: volatiles in fungal-bacterial interactions. *Front Microbiol* 6:1495. doi:10.3389/fmicb.2015.01495
- Selosse MA, Strullu-Derrien C (2015) Origins of the terrestrial flora: a symbiosis with fungi? *Bio Web Conf* 4:00009. doi:10.1051/bioconf/20150400009
- Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moëgne-Loccoz Y et al (2013) Plant growth-promoting rhizobacteria and root system functioning. *Front Plant Sci* 4:1–19. doi:10.3389/fpls.2013.00356

- Vandenkoornhuysen P, Quaiser A, Duhamel M, Le Van A, Dufresne A (2015) The importance of the microbiome of the plant holobiont. *New Phytol* 206:1196–1206
- Varma A, Bakshi M, Lou B, Hartmann A, Oelmueller R (2012) *Piriformospora indica*: a novel plant growth-promoting mycorrhizal fungus. *Agric Res* 1:117–131
- Villegas J, Fortin JA (2002) Phosphorus solubilization and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NO_3^- as nitrogen source. *Can J Bot* 80:571–576
- Villegas J, Fortin JA (2011) Phosphorus solubilization and pH changes as a result of the interaction between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NH_4 as nitrogen source. *Can J Bot* 79:865–870
- Walsh F, Duffy B (2013) The culturable soil antibiotic resistome: a community of multi-drug resistant bacteria. *PLoS One* 8:e65567. <http://dx.doi.org/10.1371/journal.pone.0065567>

Chapter 9

Arbuscular Mycorrhizal Symbiosis: Genetic and Functional Diversity

Rekha Pandey and Neera Garg

Abstract Arbuscular mycorrhiza (AM) is the most widespread plant symbiosis that improves plant productivity and resistance to nutrient stress. Numerous studies have demonstrated a high variability in the symbiotic outcome of different combinations of host plant and AM fungi. This reflects functional diversity in AM fungi in terms of variation in underlying physiological processes. The variability exists not only between isolates of different species but also within different isolates of the same species. This can be correlated to the high genetic variability observed within this group of fungi. However, little is known about the genetic diversity of AM fungi due to the strict biotrophy of these fungi, difficulties in obtaining sufficient fungal material, and to the lack of knowledge of the reproductive system and the mutation rate. Studies have shown that within the same cytoplasm, AM fungi contain thousands of nuclei and show extremely high levels of genetic variation for some loci. However, knowledge about the arrangement of this variation between, or within, nuclei remains controversial. It has been proposed that AM fungi could either be homokaryotic or heterokaryotic. In addition to genetic diversity, variability in life strategy patterns of different species could account for the functional diversity in AM symbiosis, for example, variation in the hyphal growth, rate of phosphate uptake and transfer and even in expression of specific genes. This review thus attempts to discuss the reported findings on the genetic and functional diversity within this mutualistic symbiotic association.

9.1 Introduction

Arbuscular mycorrhizal (AM) symbiosis is an association between plant roots and a specific fungal group, the glomeromycetes. This group of fungi-arbuscular mycorrhizal fungi (AM fungi; phylum Glomeromycota; Schüßler et al. 2001) are unique due to their age, lifestyle and genetic make-up. AM fungi may have evolved over 1000 million years ago and can be seen as living fossils because they have

R. Pandey • N. Garg (✉)
Department of Botany, Panjab University, Chandigarh 160014, India
e-mail: garg_neera@yahoo.com; gargneera@gmail.com

co-existed relatively morphologically unaltered with plants for more than 400 million years (Parniske 2008). The symbiosis is frequent in all early diverging lineages of the major plant clades. Non-mycorrhizal species or other mycorrhizal types developed in plant lineages of more recent origin. This suggests that this symbiosis is the ancestral form of mycorrhizal interactions and that it played a critical role in the evolution of land plants (Smith and Read 2008). They are keystone organisms that form an interface between soils and plant roots; and they are also sensitive to changes in soil and plant conditions (Giri et al. 2005). The main physiological basis for this symbiosis is usually considered to be bidirectional transfer of nutrients: the extraradical mycelium (ERM) of the fungus acts as an extension of the root system and takes up phosphate (P), nitrogen (N), sulfur and trace elements from the soil and delivers these nutrients *via* the intraradical mycelium (IRM) to the plant (Allen and Shachar-Hill 2009; Smith and Smith 2011). AM fungi may supply up to 90% of the host plant's nitrogen and phosphorus requirements (Smith and Read 2008). In exchange, they receive up to 30% of the host's photosynthate (Drigo et al. 2010; Kivlin et al. 2011). These fungi are obligate symbionts and cannot survive without this C supply (Bücking et al. 2012).

AM fungi comprise of intra- and extraradical structures. The intraradical hyphae can penetrate the outer cell wall of root and grow between or inside of the root cell wall and plasma membrane where they develop the intraradical structures, arbuscules and vesicles. The extraradical structures are hyphae and spores that develop outside of the roots in the soil. Root colonization by AM fungi is preceded by mutual recognition *via* diffusible molecules released by both symbionts, culminating in differentiation of the fungal tip of the growing hypha into a lense-shaped hyphopodium for docking at the root surface (Bonfante and Requena 2011; Nadal and Paszkowski 2013). The fungus then penetrates the outer root cell layers, spreads longitudinally within the root cortex and forms branched, tree-shaped hyphal structures called arbuscules, in cortical cells (Gutjahr and Parniske 2013). The formation of intracellular fungal structures and the extent of root colonization are dynamically regulated by the plant likely to optimize symbiotic benefit according to the plants physiological and developmental status that results from environmental conditions (Carbonnel and Gutjahr 2014). It has been reported that under high Pi supply, AM development is repressed (Breuillin et al. 2010; Balzergue et al. 2013). This suppressive effect of high Pi on root colonization by AM fungi is partially overruled by nitrogen (N) starvation and to a lesser extent by potassium, calcium or iron starvation (Nouri et al. 2014), suggesting that plants control the symbiosis in function of their nutrient requirements according to Liebig's law of the minimum (Carbonnel and Gutjahr 2014).

9.2 Diversity of AM Fungi: More Complex Than It Seems?

AM fungi are among the world's most common soil microorganisms and associate with more than 80% of vascular plant species ranging from bryophytes to tracheophytes, including many agricultural important crop species (Smith and Read 2008). It has been suggested that AM fungi play a key role in determining the distribution and abundance of plant species (van der Heijden et al. 1998, 2015). In order to understand the role of AM fungi in shaping the structure and composition of plant communities, information about their diversity and distribution is needed (Opik et al. 2013). Till date approximately 244 species have been described within the fungal phylum, Glomeromycota (Schüßler et al. 2001; Schüßler 2014; Lee et al. 2015; van der Heijden et al. 2015; Prasad et al. 2017). Molecular studies have suggested that diversity of these fungi may be much greater (Kivlin et al. 2011). In fact, high genetic variation has been reported even within different isolates of a species (Vandenkoornhuysen and Leyval 1998; Clapp et al. 2001); it has been shown that the genetic diversity in even one initial spore can be sufficient for the development of phenotypically different variants of one fungus (Ehinger et al. 2012) affecting various important functions such as colonization rates, growth of extraradical hyphae and phosphorus uptake of AM fungi giving rise to functional diversity (Munkvold et al. 2004; Avio et al. 2006; Croll et al. 2008; Angelard et al. 2010; Torrecillas et al. 2012). Genetic diversity within species or among isolates originates from the typical genetic structure of AM fungi. Hundreds or thousands of nuclei exist together within a single spore or hypha of AM fungi, meaning that the genetic structure of AM fungi is 'multigenomic' (Kuhn et al. 2001). Estimates of global AM fungal richness, based on environmental ribosomal DNA sequences, range from 341 (Opik et al. 2013) to 1600 operational taxonomic units (OTUs) (Koljalg et al. 2013) or even higher (Kivlin et al. 2011). These 300–1600 AM fungal taxa associate with approximately 200,000 plant species (Brundrett 2009), showing that host specificity must be very low (van der Heijden et al. 2015). Although AM fungi have not been found to be strictly host specific, many recent studies have indicated high functional diversity of AM fungi with different combinations of host plant and AM fungi having different effects on various aspects of symbiosis (Jansa et al. 2008; Wagg et al. 2011; Maherli and Klironomos 2012; Tian et al. 2013; Garg and Pandey 2015). There is still a lack of understanding about why a particular AM fungal isolate is much more beneficial than others although it has recently been demonstrated that both host and fungus can discriminate among their partners, reciprocally rewarding those partners that provide more mutualistic benefit (Kiers et al. 2011; Fellbaum et al. 2012; Bucking et al. 2016). Trade-offs in phylogenetically conserved traits have occurred during the evolution of the Glomeromycota lineage, affecting the growth of the fungus and plant host (Hart and Reader 2002; Powell et al. 2009). Hart and Reader (2002) demonstrated that members of Gigasporaceae family preferentially produce extraradical hyphal biomass in the soil, while members of Glomeraceae family extensively colonize roots. These trade-offs in hyphal traits contribute to higher nutrient acquisition and biomass of

plants in symbiosis with Gigasporaceae species, and greater pathogen protection of plants that associate with Glomeraceae species (Powell et al. 2009; Kivlin et al. 2011). These long-existing trade-offs may also lead to interactions between AM fungal taxa that could affect community assembly. If traits are phylogenetically conserved, competitive exclusion between closely related AM fungi could lead to a community in which species are less related than expected by chance (i.e. phylogenetic overdispersion). Alternatively, if environmental filtering or dispersal limitation selects for these traits, species within AM fungal communities could be more closely related than expected by chance (i.e. phylogenetic clustering) (Kivlin et al. 2011). The following sections therefore, explore the diversity in AM symbiosis at two levels: genetic and functional.

9.3 Genetic Diversity

While the ecological importance and evolutionary novelty of AM fungi have become clear, the basic genetics of these fungi remain enigmatic. Recent use of molecular-based methods in AM fungi studies has enabled direct identification of AM fungal species in plant roots or in soils, and it has been revealed that actual AM fungal diversity in ecosystems could be higher than expected (Gollotte et al. 2004; Kivlin et al. 2011). In addition, DNA polymorphism within AM fungal isolates by different geographic origin, even within a single spore, was identified by the use of molecular techniques (Clapp et al. 2001; Börstler et al. 2008). Stukenbrock and Rosendahl (2005) used a hierarchical design to study multilocus genotypes of three *Glomus* species. Significant genetic structure was found at a small scale, among plots separated by a few metres, whereas among neighbouring field sites, with differing agricultural treatments, no differentiation was detected. Vandenkoornhuysen et al. (2001) used inter-simple sequence repeat (ISSR) fingerprints and ribosomal gene polymorphisms to study differentiation among AM fungi from different sewage treatments in a field. A high degree of diversity was found for two *Glomus* species, and the observed diversity was structured among field plots of different treatments. Using in vitro propagated *G. intraradices* from a field population, Koch et al. (2004) found high genetic diversity and differentiation among field plots. In the same population, Corradi et al. (2007) found polymorphism in copy numbers of ribosomal genes. It has been shown that even the genetic diversity among isolates of the same phenetic species reflects diversity in development, function and symbiotic performance, thus reflecting diversity on the phenotypic level (Koch et al. 2004; Munkvold et al. 2004). Most of the knowledge on AM fungi genomes comes from *Glomus intraradices* (*Rhizophagus irregularis*) because this species can easily be cultivated in vitro. *G. intraradices* DNA harbours a low content of Guanine and Cystein (30–35% of the whole genome) (Hosny et al. 1997) against 50% for the majority of other organisms and particularly plants. This difference allows detection of plant or microorganism contaminations in the extracted DNA (Corradi et al. 2004). The size of the *G. intraradices* genome was

estimated around 16.54 megabase pairs (Mbp) by reassociation kinetics (Hijri and Sanders 2004). The DNA content per nucleus was estimated at between 0.14 picogram (pg) for AM fungi *Scutellospora pellucida* and 1.15 pg for *Scutellospora gregaria* by flow cytometry (Hosny et al. 1998). The ploidy level is unknown for most AM fungi species, although *G. intraradices* and *Scutellospora castanea* appear to be haploid, based on reassociation kinetics (Hijri and Sanders 2004).

The genomic structure of AM fungi is unusual in at least two respects. First, AM fungi are multinucleate at all stages of their life history. Individual cells may contain as many as a few hundreds to tens of thousands of nuclei depending on the fungal species and the method of analysis employed (Cooke et al. 1987; Becard and Pfeffer 1993; Hosny et al. 1998). As the AM fungal hyphae lack regular septa and the fungi do not appear to go through a uninucleate or sexual stage, the vegetative structures can be thought of as free-flowing populations of nuclei (coenocytic). The second unusual aspect of their genetics is that individual cells can have very large amounts of genetic variation, with repetitive regions such as ribosomal RNA genes (rDNA) having several genetically different copies derived from single spores (Hijri et al. 1999; Clapp et al. 1999; Pringle et al. 2000; Pawlowska and Taylor 2004). While a component of this variation has been found to be due to non-mycorrhizal fungi that cohabit with, and contaminate, AM fungi (Hijri et al. 2002), these contaminants do not negate the high diversity of rDNA of AM fungal origin (Pringle et al. 2003). Moreover, a similar level of variation within spores has been observed within single-copy regions of the genome (Kuhn et al. 2001; Pawlowska and Taylor 2004), with, for example, 13 different variants of putatively single-copy gene, DNA polymerase 1 (*PLS1*), being found within individual spores of *Glomus etunicatum*. To explain this, two basic organizational structures have been proposed (Fig. 9.1). Firstly, it is possible that all intracellular variation is present within individual nuclei and all of the nuclei within a cell are identical, i.e. *homokaryotic* (Pawlowska and Taylor 2004; Pawlowska 2005; Fig. 9.1a). Alternatively, it is possible that much of the genetic variation may be distributed between nuclei, with each cell containing multiple genomes, i.e. *heterokaryotic* (Kuhn et al. 2001; Hijri and Sanders 2005; Bever et al. 2008; Boon et al. 2015; Fig. 9.1b). Thus, the entire intrasporal rRNA variation could be either contained in every single nucleus (homokaryosis) or distributed among different nuclei (heterokaryosis) (Pawlowska 2005) (Fig. 9.1). The homokaryotic organization implies that the nuclear polymorphism reported in these organisms is the result of orthologous allelic variants partitioned between chromosomes (i.e. polyploidy) or paralogous copies within a chromosome, while in the heterokaryotic state, different allelic variants could be evenly partitioned among distinct nuclei or be present in a group of complementary nuclei. There are supporters of both the theories (Pawlowska and Taylor 2004; Hijri and Sanders 2005; Pawlowska 2005; Bever et al. 2008; Boon et al. 2015) providing evidences in their support. However, it is possible that these scenarios might not be mutually exclusive, and the genetic variation among and within AM fungi isolates is likely to be a continuum between these two states, being shaped by modest rates of hyphal fusion and segregation (Bever and Wang 2005).

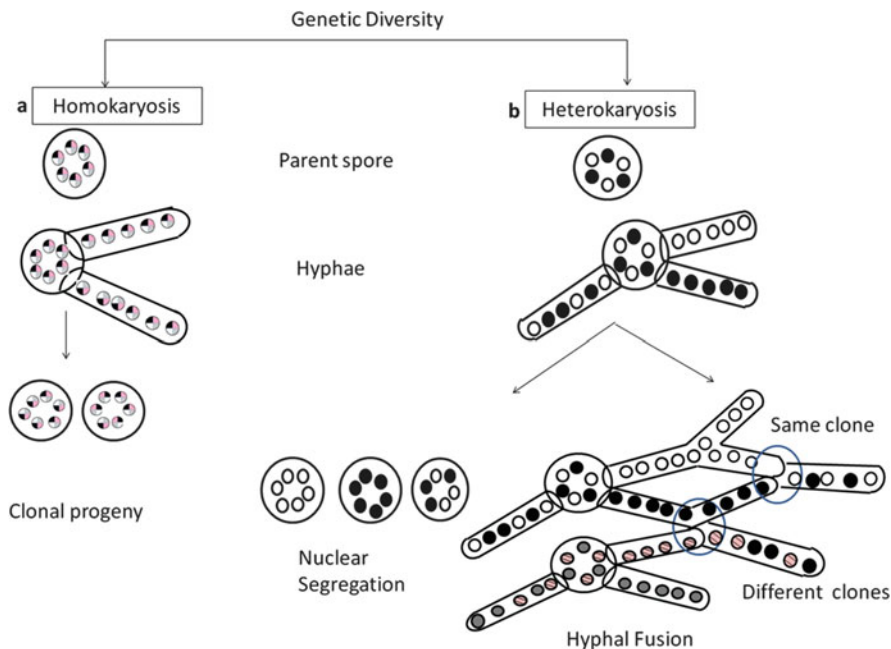


Fig. 9.1 Two alternative hypotheses on genomic organization in AM fungi: (a) Homokaryotic, where entire intrasporal rRNA variation is contained in every spore; clonal progenies in this case contain the genetic composition of parental spore. (b) Heterokaryotic, where the variation is distributed between different nuclei with each cell containing multiple genomes—under heterokaryotic nuclear organization, process of nuclear segregation and hyphal fusion could segregate and remix variation. Hyphal fusion between same hyphal clones restores genetic diversity, while between different clones creates new genetic diversity

Pawlowska and Taylor (2004) found that high levels of intracellular molecular variation within isolates of *Glomus etunicatum* were not lost due to segregation. They observed that each of 20 single progeny spores had all 13 variants of PLS1, a putatively single-copy gene. They argued that this was inconsistent with heterokaryotic organization of the genome, with their statistical confidence in this conclusion coming from simulations of the segregation process that assumed haploidy, no hyphal fusion and no selection. Instead, they proposed that all 13 variants of the PLS1 gene were present within each nucleus (and all nuclei in the hyphae were identical), with the persistence of the large number of variants within individual spores resulting from very high ploidy in these fungi (i.e. at least 13 ploids) and the suspension of gene conversion (Fig. 9.1a). This explanation came into conflict with the subsequent observation that *G. etunicatum* is actually haploid (Hijri and Sanders 2005). Bever and Wang (2005) presented a resolution to this apparent conflict by using a simulation similar to that of Pawlowska and Taylor (2004) to demonstrate that modest levels of hyphal fusion would allow remixing of the nuclei and reduce the effective rate of segregation to a level consistent with the Pawlowska and Taylor's laboratory observations. With sufficient rates of hyphal fusion, high levels

of variation can be maintained within spores over long periods of time, which is consistent with Pawlowska and Taylor field observations (Bever et al. 2008). According to Pawlowska (2005, 2007) coexistence of polymorphic rRNA gene sequences observed in individual nuclei indicate that the process of concerted evolution may have been impaired in this group of fungi during evolution. [Concerted evolution is a universal recombination-driven process responsible for rRNA gene sequence homogeneity within an individual and among individuals of a recombining population (Arnheim et al. 1980; Dover 1982). Over time, these processes can reduce variation within multicopy gene families, accounting for the low variation within rDNA gene families in other organisms (Hamby and Zimmer 1992; Avise 2004)]. A study by Stukenbrock and Rosendahl (2005) used three codominant genetic markers of confirmed AM fungi origin to estimate the genetic structure of two AM fungal populations from agricultural fields, and they did not find evidence of significant recombination. However, Bever and Wang (2005) noted that the presence of the three types within a nucleus is not a definitive test for homokaryosis as the nuclei could still vary in the numbers for each of the three ITS types as well as in other regions of the genome. Pawlowska and Taylor (2005) suggested that the changes in copy number of rDNA are not relevant, citing evidence that copy number can be dynamic within a cell cycle.

As opposed to this, proponents of the theory of heterokaryosis postulate that although AM fungi like *G. intraradices* and *G. etunicatum* are haploid as other fungi, but rRNA variation occurs between different genomes that are present in genetically different nuclei in the coenocytic mycelium (Kuhn et al. 2001; Hijri and Sanders 2005; Bever et al. 2008) (Fig. 9.1b). Heterokaryosis has been proposed to have arisen by hyphal anastomosis and accumulation of mutations (Bever and Wang 2005; Croll et al. 2009). Pawlowska and Taylor (2005) argued that there is no evidence of the level of hyphal fusion assumed in Bever and Wang's simulation, citing evidence of barriers to hyphal fusion between geographically isolated populations of *G. mosseae* (Giovannetti et al. 2003). However, this same body of work shows very high rates of hyphal fusion within isolates of many species of *Glomus* (Giovannetti et al. 2001, 2004). The simulation of Bever and Wang simply assumed that offspring from a single spore could fuse, which is exactly what had been demonstrated by the work of Giovannetti et al. (2003). Using a stochastic simulation of nuclear segregation, Bever and Wang (2005) demonstrated that modest rates of hyphal fusion can maintain high levels of nuclear variation within spores at equilibrium. While species within *Glomus* bearing small spores may have relatively small numbers of nuclei, which makes them very vulnerable to drift, *Glomus* spp. generally have high rates of hyphal fusion which will reduce the rate of genetic drift (de la Providencia et al. 2005; Voets et al. 2006). In contrast, *Scutellospora* and *Gigaspora* generally appear to have lower rates of hyphal fusion (de la Providencia et al. 2005; Voets et al. 2006), but these fungi consistently have larger numbers of nuclei with estimates ranging from a thousand to tens of thousands (Hosny et al. 1998). Croll et al. (2009) confirmed that genetically distinct AM fungi, from the same field, could anastomose, resulting in viable cytoplasmic connections through which genetic exchange can occur. These results suggest that

hyphal fusion rates are sufficient to offset the force of drift in AM fungi, potentially providing an explanation for persistence of high levels of variation in AM fungal nuclei (Bever et al. 2008). The variation that exists between nuclei would segregate as hyphae grow and divide in a process analogous to assortment during meiosis. Fusion of genetically different hyphae could remix and recombine variation in an analogous manner as fusion of gametes in sexual organisms (Fig. 9.1b). Assuming that the phenotype is a function of the nuclear composition of the hyphae, this process could mimic the creative process of sexual reproduction by bringing together novel genetic variants into the same functional organism (Bever et al. 2008). Recently, Boon et al. (2015) have cited evidences in favour of heterokarysis in AM fungi. First, they cite the evidence for within-isolate sequence polymorphism in *Rhizophagus irregularis* DAOM 197198 (synonym *Glomus irregulare*) and *Glomus etunicatum* (synonym *Claroideoglomus etunicatum*) transcripts (Boon et al. 2010; Tisserant et al. 2012). Second, segregation of genetic variation between parent and off-spring has been demonstrated for *R. irregularis* (Angelard et al. 2010) and *G. etunicatum* (Boon et al. 2013). Patterns of genetic segregation between parent and clonal offspring indicate that different fractions of genetic variation are passed on to different spores. Moreover, this variation appears to make a difference to the phenotype of the offspring isolate (Angelard and Sanders 2011). Third, within-isolate heterokarysis has been demonstrated for several loci (Boon et al. 2010). Fourth, several AM fungi taxa seem at no part of their life cycle, reduced to a single nucleus (Jany and Pawlowska 2010; Marleau et al. 2011; Ehinger et al. 2012). In 2010, Jany and Pawlowska examined the dynamics of spore nuclei in *Glomus etunicatum* using live three-dimensional imaging and mathematical models. They observed that spores of *Glomus etunicatum* are formed not by false sporogenesis (where serial divisions of a single founder nucleus occurs), but the spores are populated by an influx of a stream of nuclei from the surrounding mycelium which might account for the heterogeneity. Marleau et al. (2011) found that spores used for dispersal of AM fungi contained nuclei with two origins—those that migrate into spore from the coenocytic mycelium and those that arise by mitosis in spore which led them to postulate that probably AM fungi lack the genetic bottleneck of a single-nucleus stage at any point in the AM fungi life cycle, which sets AM fungi apart from filamentous fungi (that are heterokaryotic only in a part of their reproductive cycle) (Boon et al. 2015).

Although recent publications of the *Rhizophagus irregularis* genome (Tisserant et al. 2013) and single-nucleus sequencing (Lin et al. 2014) have reported evidence in favour of homokaryosis, it is unclear whether the approach adopted in these studies is sufficient to provide a definite answer to the debate. In a latest report, Boon et al. (2015) have addressed the question of the extent of genome differentiation and its physical partitioning in *Rhizophagus*. They found evidence for genome differentiation within the *Rhizophagus* cytoplasm, both genome-wide and on the scale of a single locus which led them to propose that this population of partly heterogeneous genomes in *Rhizophagus* is analogous to a pan-genome, since there may not be one typical genome within an isolate representative of all the other but rather a population of partly differentiated genomes. They cite four observations

to support this interpretation. First, for several AM fungi, it has been shown that they are at no point in their life cycle reduced to a single genome as stated earlier (Jany and Pawlowska 2010; Marleau et al. 2011; Boon et al. 2013). Second, *Rhizophagus* spores do not germinate below a certain number of nuclei per spore, which is roughly 65 nuclei for *R. irregularis* (Marleau et al. 2011). Third, for *R. irregularis* and *G. etunicatum*, it has been shown that genetic polymorphism is expressed in the transcriptome (Boon et al. 2010; Tisserant et al. 2012), which indicates that differentiation at the genome level could play a role in the functioning of *Rhizophagus* isolates. Finally, the high amounts of genetic variation in AM fungi isolates have been proposed to play a role in the ability of AM fungi to adapt to a wide range of host plants (Angelard et al. 2010). However, basic parameters can differ substantially between different members of the Glomeromycota (Gianinazzi-Pearson et al. 2012)—their genome sizes vary, not much is known about the genome structure except the model species—*G. intraradices* and retrotransposons have been suggested to play an important role in the genome of at least one species (*Scutellospora castanea*; Gollotte et al. 2006). Not only that, anastomosis has not been observed in Gigasporaceae and other lineages, suggesting a completely different of the mycelium in such cases (Purin and Morton 2011). Also in contrast to *G. intraradices*, *G. mosseae* has been shown to have a rather uniform worldwide population structure suggesting a different genetic disposition (Gianinazzi-Pearson et al. 2012).

Despite recognition of the importance of genetic diversity of AM fungi, little is known about its role in ecosystems. It has been demonstrated that genetically different AM fungal isolates, even from the same species, have different effects on their host plants (Avio et al. 2006; Koch et al. 2006). Recently, Colard et al. (2011) observed that genetically different AM fungal isolates could differ in their ability for survival or functionality in their host plants. This supports the view that genetic variation could lead to functional diversity of AM fungi in ecosystems.

9.4 Functional Diversity

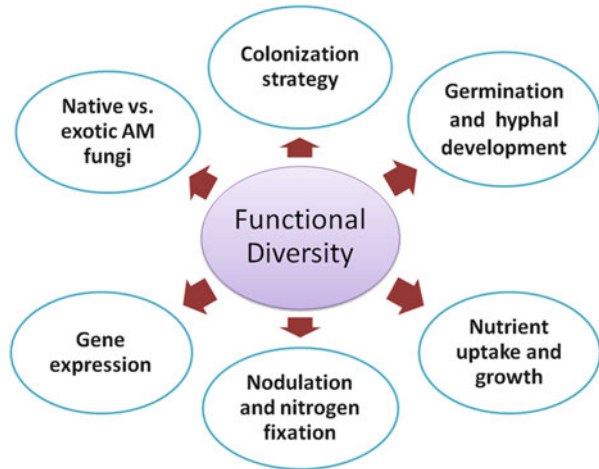
The relationship between arbuscular mycorrhizal (AM) fungi and their plant hosts is considered as a classic example of a reciprocally beneficial mutualism; both partners benefit from the symbiosis, with plants providing carbohydrates to their fungal partners and fungi providing mineral nutrients, such as nitrogen (N) and phosphorus (P) to their host plants. The presence of AM in virtually all terrestrial habitats (Smith and Read 1997; Brundrett 2004) together with the hitherto, comparatively small number of identified AM fungi taxa (244, Schüßler 2014; van der Heijden et al. 2015), could indicate a high promiscuity among the fungal species, and it was long believed that most AM fungi species are able to form a successful symbiosis with most plant hosts and are not host specific. However, recent studies have clearly brought out the host preference in AM fungi, thus emphasising the need for selecting efficient AM fungi for inoculating a particular host. This

variation has been observed among AM fungi isolates belonging to different species, as well as among isolates of the same species (van der Heijden et al. 1998; Klironomos 2003; Munkvold et al. 2004; Smith et al. 2004; Burleigh et al. 2002; Lerat et al. 2003a, b; Avio et al. 2006; Jansa et al. 2008; Wagg et al. 2011; Maherali and Klironomos 2012; Tian et al. 2013; Pellegrino and Bedini 2014). Antunes et al. (2011) even found evidence for functional diversity in AM fungi originating from contrasting climates. Many reports have stated the higher efficiency of *G. intraradices* (= *R. irregularis*) over other AM fungi (Avio et al. 2006; Peng et al. 2011; Tufenkci et al. 2012). As opposed to this, many reports have shown *Funneliformis mosseae* to be more beneficial (Stancheva et al. 2006; Song et al. 2012; Huang et al. 2013). The AM fungi species commonly used in functional studies, e.g. *Glomus intraradices* and *Glomus mosseae* from the Glomeraceae or *Gigaspora margarita* from the Gigasporaceae, dominate almost all biodiversity studies even across drastically different environments. Functional studies therefore often contain a certain bias towards fungal species that either confer a clear benefit on their host plant or are highly abundant and therefore easily detected in culture systems (Feddermann et al. 2010; Opik et al. 2013). However, there are numerous examples of negative effects of AM fungi on host-plant growth (Johnson et al. 1997; Klironomos 2003; Li et al. 2008). In a recent study, de Novais et al. (2014) evaluated the functional variability of 41 isolates of 20 species and eight genera of AM fungi for root colonization, growth promotion and P uptake of corn and observed the relationship of this functional variability with the isolates genetic variability revealed by PCR-RFLP analysis. All the isolates abundantly colonized the corn roots but only 23 promoted higher shoot dry mass and P leaf content. A functional variability was observed between isolates of distinct species and isolates of the same species showing no correlation between the ability to promote growth with the genus, species or the origin of the isolates. Cluster analysis based on functional variability data separated the isolates *Acaulospora morrowiae* (Am2), *Acaulospora* sp. (Aca), *A. colombiana* (Ac3, Ac4 and Ac5), *Gigaspora albida* (Gia1), *Gi. margarita* (Gim4 and Gim5), *Gi. rosea* (Gir), *Rhizophagus clarus* (Rc2, Rc3, Rc4, Rc5 and Rc6), *Claroideoglomus etunicatum* (Ce4), *R. manihotis* (Rm), *Scutellospora calospora* (Sc), *S. heterogama* (Sh2, Sh3, Sh4 and Sh5) and *S. pellucida* (Sp3) from the others at the distance of 80% functional similarity. These were considered efficient in promoting functional symbiosis in corn, while the other isolates were considered inefficient. The functional diversity of the AM fungi could be manifested at various levels from colonization strategies to the induction of specific genes in the host (Fig. 9.2) each of which is discussed in detail.

9.4.1 Colonization Strategy

AM fungi form a number of different infective propagules that are used to form new mycorrhizal associations. These are spores, extraradical hyphae and infected roots.

Fig. 9.2 Different levels at which functional diversity in AM symbioses are displayed by different AM fungi



However, not all fungi are equally capable of colonizing roots with all of the above-mentioned propagules, and there is conflicting evidence of major differences in colonization strategy between members of the Glomineae and Gigasporineae. Abbott et al. (1994) showed that mycorrhizal root pieces were effective propagules for *Glomus* and *Acaulospora* isolates but not for *Scutellospora* isolates. Biermann and Linderman (1983) reported a similar result. They examined the role of root fragments as propagules and found high infectivity from those containing *Glomus* and *Acaulospora* species, but none from root fragments containing *Gigaspora* species. They attributed this difference to the presence of vesicles. Later, Klironomos and Hart (2002) tested the abilities of eight fungal species from four different genera to colonize roots using three different types of inoculum. *Glomus* and *Acaulospora* isolates colonized from all inoculum types, whereas *Gigaspora* and *Scutellospora* isolates colonized mainly from spores and to a limited degree from root fragments. Extraradical hyphae were not suitable propagules for the species of *Gigaspora* and *Scutellospora* tested. This clearly highlighted that AM fungi differ on the basis of different colonization strategies.

9.4.2 Germination of Spores and Hyphal Development

The development of AM fungi prior to root colonization, known as presymbiosis, consists of three stages: spore germination and hyphal growth, host recognition and **appressorium** formation. Spores of the AM fungi are thick-walled multinucleate resting structures. The germination of the spore does not depend on the plant, as spores have been germinated under experimental conditions in the absence of plants both **in vitro** and in soil. However, the rate of germination can be increased by host root **exudates** (Douds and Nagahashi 2000). AM fungal spores germinate given

suitable conditions of the soil matrix, temperature, carbon dioxide concentration, pH and phosphorus concentration. AM fungi recognize and respond to their potential hosts, whereas the presence of non-host plants has no stimulatory effect on hyphal growth or is even inhibitory (Requena et al. 2007). AM fungal colonization of host roots involves a series of events that are tightly regulated by both partners. Recognition and the subsequent initiation of the symbiotic program in the AM fungi and potential host plant could be described as compatibility and is genetically predetermined. According to Giovannetti et al. (2003), spores and/or sporocarps of the six *G. mosseae* isolates from different geographical areas, showed different germination abilities, depending on the experimental system. Surface-sterilized spores grown in vitro exhibited the lowest germination percentages in all isolates, and some of these (BEG25, IN101C and SY710) did not germinate at all in axenic culture. In contrast, spore germination was higher in all isolates when the in vivo culture system was used. Hyphal growth per germinated spore, assessed in vivo, varied with the different isolates and ranged from 34.5 ± 3.5 mm and 35.9 ± 2.9 in BEG69 and SY710, respectively, to 119.5 ± 14.4 mm in IMA1. Within the root, fungal hyphae grow intercellularly until they reach the inner cortex where they penetrate cortical cell walls and form characteristic intracellular hyphal structures (Genre et al. 2008). A varied range of structures is formed by AM fungi in the roots of plants, as first highlighted by Gallaud (1905) such as hyphal coils, arbuscules and intermediate structures involved in nutrient transfer (Smith and Smith 2012). Within the mycorrhizal roots, different types of hyphal morphology can be identified. Depending on the type of mycorrhiza, characteristic, highly branched arbuscules (Arum-type) or heavily curled “coils” (Paris-type) (Smith and Read 1997) are developed, although there is a continuum of intermediate structures (Dickson 2004). The colonization morphology depends on the combination of the plant and fungal species (Feddermann et al. 2010). In general, AM fungi of the Glomeraceae usually form the Arum-type of mycorrhiza (Cavagnaro et al. 2001; Burleigh et al. 2002; Dickson 2004; Feddermann et al. 2008), while other genera, e.g. Gigasporaceae, form Arum-type AM or intermediate types of AM with Paris-type hyphal coils (Cavagnaro et al. 2001; Dickson 2004; Karandashov et al. 2004; Smith et al. 2004; Dickson et al. 2007). The extraradical mycelium (ERM) plays critical roles in uptake and rapid translocation of nutrients to the intraradical structures and in foraging to locate new roots on the same or different plants, which are new sources of organic C (Smith and Smith 2012). The ability to develop extensive and highly interconnected extraradical mycorrhizal networks could represent an important feature of efficient AM fungi. Recently, the genetic diversity, i.e. the genetic composition, of the coenocytic ERM has also been discussed as being an important factor in the recognition process (Koch et al. 2006; Croll et al. 2009). Some studies have provided data on the large diversity among different AM fungal isolates in the extension, viability, structure and anastomosis formation ability of ERM originating from mycorrhizal roots (Abbott and Robson 1985; Hamel et al. 1990; Friese and Allen 1991; Giovannetti et al. 2001). Avio et al. (2006) investigated the functional significance of extraradical mycorrhizal networks produced by geographically different isolates of the AM fungal species

Glomus mosseae and *Glomus intraradices* and detected a large functional diversity between the two, since *G. intraradices* isolates were generally more effective than *G. mosseae* isolates. They observed not only interspecific but also intraspecific functional diversity, both in *G. mosseae* and in *G. intraradices*. In particular, AM fungal isolates producing higher total hyphal lengths and densities yielded larger increases in total shoot biomass, confirming that the growth ability and developmental pattern of ERM are important factors of fungal efficiency (Jakobsen et al. 1992). Plant P content also correlated positively with hyphal length, which could be responsible for overall functional diversity (Avio et al. 2006). Mycelia produced by different fungi have quite varied characteristics, in terms of hyphal diameters (usually in the range of 2–20 μm), extent of growth away from the root and ability to absorb nutrients at a distance [up to 25 cm (Jansa et al. 2003)] and translocate them to the root (Jakobsen et al. 1992; Smith et al. 2000; Drew et al. 2003; Munkvold et al. 2004). Many AM fungi produce runner hyphae of relatively large diameter that can subtend tufts of finely branched hyphae; the latter turn over rapidly and are probably involved in nutrient uptake (Bago et al. 1998). Hyphal length densities in soil associated with plants in pot experiments are variable and usually in the range of 1–40 m g^{-1} depending at least in part on the identity of the AM fungus (Jakobsen 1999; Munkvold et al. 2004). Smith et al. (2004) investigated structural and functional diversity in arbuscular mycorrhizal (AM) symbioses involving three plant species and three AM fungi and measured contributions of the fungi to P uptake using compartmented pots and ^{33}P . They observed that flax (*Linum usitatissimum*) responded positively to all fungi, and medic (*Medicago truncatula*) to *Glomus caledonium* and *G. intraradices*, but not *Gigaspora rosea*. Tomato (*Lycopersicon esculentum*) showed no positive responses. Not only host genotype but AM fungal identity also influenced the outcome of the association. Hyphal growth in soil was very low for *Gi. rosea* and high for both *Glomus* spp. Hypha lengths in root + hyphal compartment (RHC) and hyphal compartment (HC) were similar for *G. intraradices* but much higher in HC for *G. caledonium*. In order to evaluate host plant performance relative to different soil arbuscular mycorrhizal fungal (AM fungi) communities, *Andropogon gerardii* seedlings were grown with different AM fungi communities (Gustafson and Casper 2006). The communities consisted of spores of *Glomus etunicatum* and *Glomus intraradices*. There was no difference in root AM fungi colonization rates between single species communities of either *G. etunicatum* or *G. intraradices*, but *G. intraradices* enhanced plant growth and *G. etunicatum* did not.

9.4.3 Nutrient Uptake and Growth Response

In the context of nutrient uptake in AM symbiosis, the soil-root interface provides the direct pathway (DP), in contrast to the mycorrhizal pathway (MP). The latter involves uptake by the ERM and rapid translocation to the IRM. Delivery is followed by nutrient export from the fungus across the interfacial apoplast to the

plant. Positive mycorrhizal growth responses arise largely from increased P uptake via the MP, alleviating P deficiency, but can also come from increased uptake of other growth-limiting nutrients (Smith and Smith 2012). However, the contribution of the plant or mycorrhizal pathway to total P uptake also depends on the plant and fungal species. Important functional differences in terms of P acquisition strategies have been recognized among AM fungi species and also among AM fungi isolates belonging to the same species. These are mainly expressed as (1) morphological traits such as the ability (rate and extent) of the AM fungi to colonize the root and the soil and (2) physiological traits that mainly include the efficiency of the mycorrhizal pathway to take up the P from the soil solution, transport and deliver it to the roots, along with the carbon requirement from the plant host (van der Heijden and Scheublin 2007). There is a consensus (Avio et al. 2006; van der Heijden and Scheublin 2007) that the differential increases in P supply to host plants are mainly attributed to morphological and physiological properties of the mycorrhizal extraradical mycelium (ERM). However, recent work by Mensah et al. (2015) demonstrated that the greater effect of some AM fungal isolates on plant P and N nutrition was more likely the result of more efficient P and N uptake systems and/or higher nutrient transport rates to the host than the length of ERM. This is consistent with other studies in which no correlation between the dimensions of the ERM and P uptake and/or MGR (mycorrhizal growth responsiveness) was found (Hart and Reader 2002; Smith et al. 2000). A meta-analysis recently revealed that the mycorrhizal colonization is only in part responsible for the high diversity in MGR that can be observed but that AM fungal taxa also differ in their mycorrhizal benefit per unit root length colonized (Treseder 2013). Thonar et al. (2011) quantified differences in P acquisition and use efficiency of medic (*Medicago truncatula*), when colonized by three different AM fungi species (*Glomus intraradices*, *Glomus claroideum* and *Gigaspora margarita*) using dual radioisotope labeling (^{32}P and ^{33}P): ^{33}P labeling determined hyphal P uptake from different distances from roots over the entire growth period, whereas ^{32}P labeling investigated hyphal P uptake close to the roots over the 48 hours immediately prior to harvest. *G. intraradices*, *G. claroideum* and *Gi. margarita* were able to take up and deliver P to the plants from maximal distances of 10, 6 and 1 cm from the roots, respectively. *G. intraradices* most rapidly colonized the available substrate and transported significant amounts of P towards the roots but provided the same growth benefit as compared to *G. claroideum*, whose mycelium was less efficient in soil exploration and in P uptake and delivery to the roots. *Gi. margarita* provided low P benefits to the plants and formed dense mycelium networks close to the roots where P was probably transiently immobilized. Based on numerical modelling, they concluded that high external hyphal production at the root-fungus interface together with rapid hyphal turnover as important factors governing hyphal network development by *Gigaspora*, whereas nonlinearity in apical branching and hyphal anastomoses was key features for *G. intraradices* and *G. claroideum*, respectively. Similarly, Veresoglou et al. (2011) also observed that *G. intraradices*-inoculated *Plantago lanceolata* plants had 27.8% and 40.8% more total N and 55.8% and 23.3% more total K when compared to *Gigaspora margarita*-inoculated

counterparts in a native, nutrient limited, coastal dune soil. *G. intraradices* inoculated and non-mycorrhizal plants generally exhibited N:P:K ratios indicative of P limitation, whereas for *Gi. margarita* mycorrhizal plants, corresponding ratios strongly implied either N or K limitation. Plant P transporters that are involved in the uptake *via* the plant pathway are downregulated in response to the AM symbiosis (Chiou et al. 2001; Grunwald et al. 2009), while mycorrhiza-specific transporters that are involved in the P uptake from the mycorrhizal interface are induced (Harrison et al. 2002; Xu et al. 2007; Paszkowski et al. 2002). However, this effect can be largely species specific. *Glomus intraradices* has been shown to suppress the expression of plant P transporters of the plant pathway the most, whereas *G. mosseae* had the least effect (Grunwald et al. 2009). In tomato, almost 100% of the plant's P was taken up by *G. intraradices* via the mycorrhizal pathway, but the contribution of *Gigaspora rosea* to total P uptake was much lower (Smith et al. 2003). Wu et al. (2011) found that the benefits of AM fungi on nutrient uptake in peach were better in *G. mosseae* treatment when compared to *G. versiforme* and *Paraglomus occultum* treatments. A high functional diversity in nutritional benefit, not only among different fungal morphospecies but also among isolates within one morphospecies, has been observed (Pellegrino et al. 2011; Tian et al. 2013; Pellegrino and Bedini 2014). Recently, the effect of 31 different isolates from 10 AM fungal morphospecies on the P and nitrogen (N) nutrition of *Medicago sativa* and the P allocation among different P pools was examined by Mensah et al. (2015). The results of these investigations revealed that plant growth benefit was positively correlated to the mycorrhizal effect on P and N nutrition. A high variability in the mycorrhizal growth response (MGR) across AM fungal isolates was detected. The per cent increase in total plant biomass ranged from $7.3 \pm 10.8\%$ in plants colonized with *R. irregulare* (not significantly higher than the controls) to $207.4 \pm 36.4\%$ in plants colonized with *Acaulospora colombiana*. They divided the different isolates into high, medium and low performance isolates based on increase in total plant biomass relative to controls. The high performance isolates increased plant biomass by as much as 170% and contributed substantially to both P and N nutrition, whereas the effect of medium performance isolates particularly on the N nutrition of the host was significantly lower (18–170%). Of all the AM fungal species tested, the four *Rhizophagus* isolates led to the lowest MGR (average of $20.2 \pm 9.3\%$) and the plants did not differ in their biomass from the non-mycorrhizal controls. The high performance isolates belonged to different morphospecies and genera, indicating that the ability to contribute to P and N nutrition is not conserved and is widespread within the Glomeromycota, and differences in symbiotic performance and P metabolism are not specific for individual fungal morphospecies. Garg and Pandey (2015) demonstrated that higher beneficial effects of *R. irregularis* over *F. mosseae* in pigeon pea-AM associations were related to its higher P and N acquisition traits as well as more favourable K^+/Na^+ ratios. According to a meta-analysis study by Augé et al. (2014), AM-induced increases in root K^+/Na^+ ratio ranged from 12 to 107 percent based on AM taxa, and this heterogeneity was significant—*R. intraradices* had the largest effect on root K^+/Na^+ (average increase of 107%), followed by *F. mosseae* (45%) and *R. clarus*

(17%). More recently, Kohl and van der Heijden (2016) demonstrated that the effects of different AM fungi on nitrogen leaching varied depending on host plant species and the identity of the AM fungal species present in soil, using experimental microcosms with two different host plants (the grass *Lolium multiflorum* or the legume *Trifolium pratense*) and three different AM fungal species (*Claroideoglossum claroideum*, *Rhizoglossum irregulare* and *Funneliformis mosseae*). Their results show that the differential effects were, at least in part, explained by species-specific differences in root colonization. The AM fungus with the highest levels of root colonization (Ri) had the strongest effects on plant biomass [resulting in the greatest growth stimulation (1170%) for the mycotrophic plant species (*Trifolium*) and the greatest growth suppression (18%) for the grass species (*Lolium*)]. This was in confirmation with an earlier study by Verbruggen et al. (2012) who demonstrated that the abundance of specific AM fungal taxa, as determined by terminal-RFLP, correlated well with plant productivity and PO_4^{3-} leaching from microcosms. Thus, together, these studies showed that the AM fungal composition can influence nutrient leaching in soil.

9.4.4 Nodulation and Nitrogen Fixation in Legumes

It is also important to study the interaction of *Rhizobium* with different AM fungal species/isolates since such interactions may be relevant to N_2 fixation and to nutrient and water uptake by the legume plants. In a study using chickpea plants, the symbiotic efficiency was found to be dependent on the particular combination of the *Rhizobium* strain and *Glomus* species, indicating selective and specific compatibilities between the bacterial strain and fungal isolate (Ruiz-Lozano and Azcon 1993). Geneva et al. (2006) evaluated the response of pea (*Pisum sativum* cv. Avola) to AM species *Glomus mosseae* and *Glomus intraradices* and *Rhizobium leguminosarum* bv. *viceae*, strain D 293, regarding the growth, photosynthesis, nodulation and nitrogen fixation activity. Their results demonstrated that the dual inoculation of pea plants significantly increased the plant biomass, photosynthetic rate, nodulation and nitrogen fixation activity in comparison with single inoculation with *Rhizobium leguminosarum* bv. *viceae* strain D 293. The effectiveness of coinoculation with *Rhizobium leguminosarum* and *Glomus mosseae* was higher at the low phosphorus level, while the coinoculation with *Glomus intraradices* appeared to be the most effective at higher phosphorus level. Assessment of comparative effects of three AM fungi species, *Glomus intraradices*, *Acaulospora tuberculata* and *Gigaspora gigantea*, was combined with cultivar specific *Bradyrhizobium japonicum* (CSBJ) in soybean cultivars on nodulation, plant growth and seed yield by Meghvansi et al. (2008) revealed that amongst the single inoculations, *G. intraradices* produced the largest increases in the parameters studied followed by *A. tuberculata* and *G. gigantea* indicating that plant acted selectively on AM symbiosis. The dual inoculation with AM fungi and CSBJ further improved these parameters demonstrating synergism between the two

microsymbionts. Among all the dual treatments, *G. intraradices* + *B. japonicum* brought about the largest increases in the studied characteristics particularly in seed weight per plant that increased up to 115.19%, which suggested that a strong selective synergistic relationship existed between AM fungi and *B. japonicum*. More recently, tripartite symbiosis of common bean (*Phaseolus vulgaris* L.) recombinant inbred line (RIL) 147 with rhizobia and three AM fungal species—*Glomus intraradices*, *Gigaspora rosea* and *Acaulospora mellea*—was assessed in sand culture by comparing the effects on the mycorrhizal root colonization, rhizobial nodulation, plant growth and phosphorus use efficiency (PUE) for symbiotic N₂ fixation by Tajini and Drevon (2012). They found that although *Glomus intraradices* well-colonized the roots of RIL147 plants, *Gigaspora rosea* and *Acaulospora mellea* colonized the roots weakly. Significant differences among colonization and nodulation of the roots and growth were found between AM fungal species—significantly more nodules were encountered for plants inoculated by *Glomus* than other AM fungal species—even nodular dry mass was higher in these plants. In addition, the combined inoculation of *Glomus* and CIAT899 strains resulted in significantly higher N and P accumulation of common bean plants and improved PUE compared with their controls. Recently, Garg and Pandey (2016) observed higher nodulation and nitrogen fixation in pigeon pea plants inoculated with *R. irregularis* as compared to *F. mosseae* which has been correlated to higher AM colonization percentage as well as higher trehalose turnover in the nodules of these plants.

9.4.5 Gene Expression

It has been suggested that differences in AM compatibility reflect differences in plant and fungal gene expression (Feddermann et al. 2010). Genetic variation caused by the composition of hyphal nuclei is important in mutual recognition of AM symbiosis. In addition, genetically different isolates of AM fungi could affect colonization strategy and mycorrhizal morphology of the plant (Koch et al. 2006; Lee et al. 2015). Koch et al. (2006) showed that genetically different *Glomus intraradices* isolates from one AM fungi population significantly alter plant growth in an axenic system and in greenhouse experiments. Isolates increased or reduced plant growth meaning that plants potentially receive benefits or are subject to costs by forming associations with different individuals in the AM fungi population. This suggested that genetic diversity of AM fungi plays a pivotal role in host-plant fitness. Croll et al. (2008) also reported a strong preference for AM fungal genotype by host plants in his experiment. Angelard et al. (2010) used genetically different AM fungal isolates of *G. intraradices* to promote the growth of rice and found that specific AM fungal genotypes could increase the biomass of rice up to five times compared with other isolates. Recent gene expression studies on plants interacting with AM fungi from different taxonomic groups have showed a partial overlap in the gene expression patterns after colonization of fungi of the Glomeraceae,

Diversisporaceae and Gigasporaceae. In an early study using array techniques, Liu et al. (2003) showed that a high number of AM-specific genes are induced in their host plants. Transcriptional analysis dissecting the common symbiosis dependent and independent signalling in rice revealed that the symbiosis signalling pathway is conserved in angiosperms (Gutjahr et al. 2008). Despite the apparent similarities in plant transcriptional responses to AM fungi, a large number of genes found in recent studies show significant variation in gene expression levels. The symbiotic phenotype in the presumed *myc*⁻¹ tomato mutant *rmc* (Barker et al. 1998) differs depending on the AM fungi against which it is challenged. The Paris-type of AM formed by *S. calospora* in tomato induced high levels of a number of defence-related genes, whilst the Arum-type of AM formed by *G. intraradices* did not induce these defence reactions (Gao et al. 2004). Hohnjec et al. (2005) reported that the gene expression pattern was similar in infections by two AM fungi species but that some genes were expressed more in specific host plants, meaning that mycorrhizae-specific gene expression was affected by the combination of host plant and AM fungi species (Lee et al. 2015). Marulanda et al. (2003) have shown that *Glomus intraradices* is one of the most efficient fungi in improving plant-water uptake in lettuce plants, while *Glomus mosseae* showed a reduced ability for the same. These differences have been related to the different regulation of plant *PIP* aquaporin genes by the fungi. Up-regulation of the *PIP* gene expression induced by *G. intraradices* enhances the water uptake of root and the root water movement. AM-inducible plant Pi transporters are involved in the acquisition of Pi released by the AM fungus at the symbiotic interface and can be used as markers for the symbiotic Pi uptake pathway (Grace et al. 2009). It has been observed that different AM species or isolates have varying influence on the expression of Pi transporters in plant species such as *M. truncatula* and tomato (Burleigh et al. 2002; Poulsen et al. 2005; Tian et al. 2013). Tian et al. (2013) studied the functional diversity of different AM fungal species (*Glomus deserticola*, *Glomus intraradices*, *Glomus mosseae*, *Gigaspora gigantea*) in influencing the expression of Pi transporters in maize roots. The expression patterns of the two genes (*ZEAmA:Pht1;3*, Pi starvation inducible, and *ZEAmA:Pht1;6*, AM inducible) were quantified using real-time, reverse transcription polymerase chain reaction (real-time RT PCR). It was observed that expression of the two genes differed with inoculation treatment, and increasing the diversity of AM fungi in maize roots led to greater expression of *ZEAmA:Pht1;6* as well as Pi uptake in shoots. The percent root colonized by *Gigaspora gigantea* was significantly lower than the other four AM fungal inoculations. All AM fungal inoculations significantly increased the expression level of the AM-inducible Pi transporter (*ZEAmA:Pht1;6*) and decreased the Pi starvation-inducible Pi transporter (*ZEAmA:Pht1;3*) in maize roots. The expression of *ZEAmA:Pht1;6* was higher in *G. mosseae* or *G. intraradices* compared to *G. deserticola* or *Gigaspora gigantea*, while the greatest repression of genes occurred in roots colonized by *G. mosseae*, *G. deserticola* and the AM mix. This also suggested that AM-inducible Pi transporter genes can be used as effective markers for a functional mycorrhizal Pi uptake pathway in plants (Poulsen et al. 2005; Javot et al. 2007). Recently, Estrada et al. (2013a, b) studied the expression of

different ion transporters and genes involved in water uptake in maize grown under saline conditions and demonstrated that the differential expression of *ZmAKT2*, *ZmSOS1* and *ZmSKOR* genes as well as chaperone and aquaporin genes (*GintBIP*, *Gint14-3-3* and *GintAQP1* genes) in maize plants led to the increased salt tolerance of the plants inoculated with *Glomus intraradices* collected from saline soil as compared to *G. intraradices* from the collection.

9.4.6 Native Vs. Exotic AM Fungi

As opposed to the introduction of new AM fungal species in an ecosystem, the use of an inoculum based on locally sourced AM fungi may be a more suitable choice because of its better adaptation to the prevailing conditions (Lambert et al. 1980), and also it would avoid the ecological risks of the introduction of foreign species (Schwartz et al. 2006). Several studies have shown higher or similar plant growth and nutritional performances of locally sourced AM fungi as compared to foreign selected ones (Requena et al. 2001; Klironomos 2003; Caravaca et al. 2003; Tchabi et al. 2010; Pellegrino et al. 2011; Estrada et al. 2013a, b; Pellegrino and Bedini 2014). Klironomos (2003) tested the effect of multiple AM fungi isolates from native and non-native sources on the mycorrhizal plant-growth responses for a number of grassland species. He found that plant growth associated with AM fungi that naturally co-occurred with a species (native AM fungi treatment) ranged from highly parasitic to highly mutualistic, depending on the combination of plant and fungal species. Calvente et al. (2004) compared the effect of native strains of AM fungi (*G. intraradices* BEG 123, *G. mosseae* BEG 124, *G. clarum* BEG 125 and *G. viscosum* BEG 126) with two non-native AM fungi (*G. intraradices* and *G. mosseae*) on olive plants and observed that the native strains of *G. intraradices* and *G. viscosum* were most effective in improving the growth of two varieties of olives. Similarly, when Williams et al. (2013) treated rooted cuttings of an endemic New Zealand tree species (*Podocarpus cunninghamii*) and an exotic and invasive grass (*Agrostis capillaris*) with an indigenous, pot-cultured AM fungi (*Acaulospora laevis*) and an exotic commercial AM fungi product (*Glomus* spp.), they observed significant increases in plant growth rates and tissue concentrations of both nitrogen and phosphorus upon inoculation with indigenous AM fungi, while the commercial AM fungi had either no effect or a negative effect on host growth and nutrient levels. Sharma et al. (2009) studied the effect of *G. geosporum*, *G. microcarpum* and a native consortium of AM fungi on post-transplanting performance of 'in vitro' raised *Curculigo orchioides* plantlets and reported plantlets inoculated with the native consortium of AM fungi consistently performed better in terms of biomass accumulation and nutrient uptake. Pellegrino and Bedini (2014) evaluated the effectiveness of the inoculation of locally sourced and foreign/exotic AM fungi on chickpea (*Cicer arietinum* L.), cultivated under a rainfed low-input system. The foreign/exotic AM fungi *Funneliformis mosseae* and *Rhizophagus irregularis* were used as single and dual species inocula. Better overall yield performances of

chickpea inoculated with the local inoculum were recorded compared to the foreign/exotic AM fungi inocula.

In contrast, there are also reports where non-native AM fungi isolates have provided greater benefits to the hosts than the native ones (Requena et al. 2001; Tian et al. 2004; Schreiner 2007). Requena et al. (2001) found that inoculation with the exotic AM fungi *Glomus intraradices* promoted faster growth of *Anthyllis* than inoculation with a mixture of native AM fungi during the first year after seedling transplanting in a degraded semiarid area. Higher effectiveness of non-native isolate of *G. mosseae* over native AM fungi mix (*Glomus mosseae*, *Glomus intraradices* and *Scutellospora calospora* isolated from Jory soil) in promoting growth and nutrient uptake in 'Pinot noir' grapevine cuttings, growing in Jory soils, was also evidenced by Schreiner (2007). More recently, Garg and Pandey (2015, 2016) have found higher benefits with the exotic single isolates of *R. irregularis* and *F. mosseae* over the native saline mix isolated from saline soils in pigeon pea plants growing under salt-stressed conditions.

9.5 Different Strategies Driving Functional Diversity In AM Fungi

The functional diversity of AM fungi has been linked to different life history strategies employed by AM fungi species (Boddington and Dodd 1999; Thonar et al. 2011; Maherali and Klironomos 2012). For example, AM fungi species differ in the amount of carbon they extract from their host (Zhu and Miller 2003; Li et al. 2008; Olsson et al. 2010), their ability to acquire phosphorus (P) (Smith et al. 2000; Drew et al. 2003) and their nutrient-storage strategies (Kiers et al. 2011). These differences in life-history strategies likely dictate the nature of competition inside and outside the host. Specifically, AM fungi strains will compete intraradically for host-derived carbon (Herrera Medina et al. 2003) but also extraradically for available mineral nutrients (Johnson et al. 2003; Parniske 2008). The classification of AM fungi into functional groups or on the basis of their life-history strategy (LHS) has become a major field of interest in the last few years (Hart et al. 2001; van der Heijden and Scheublin 2007). The LHS concept aims to explain how organisms invest their resources under perturbed (i.e. unpredictable) or stable (i.e. predictable) environments. This positions organisms on an r-K continuum with the "r" endpoint representing a quantitative and the "K" endpoint representing a qualitative extreme (Pianka 1970; Begon et al. 1996). The K-strategists are organisms that evolved traits to enhance survival in stable and predictable environments where competition is high. K-strategists principally allocate resources to growth and enhanced survival (Pianka 1970; Begon et al. 1996). The r-strategists invest their energy mainly in the production of many offspring and evolved traits that are favoured in unstable environments. Life-history patterns have been determined by the amount of resources allocated to growth, survival and reproduction over time (Pianka 1970;

Begon et al. 1996). Based on these traits, it was suggested that AM fungi belonging to the Gigasporaceae resemble the LHS of K-strategists (de Souza et al. 2005). In contrast, Glomeraceae and in particular *Glomus* species show an opportunistic behaviour, similar to r-strategists (Sykorova et al. 2007). Ijdo et al. (2010) examined the effect of repeated defoliation of in vitro grown barrel medic (*Medicago truncatula*) on the spore and auxiliary cell (AC) production dynamics of a presumed r-strategist (*Glomus intraradices*) and a presumed K-strategist (*Dentiscutata reticulata*). Decreasing the host plant's photosynthetic ability (e.g. through defoliation, shading or reducing the number of hours of daylight) reduces AM fungal colonization of the root as well as spore production in the extraradical mycelium (Daft and El Giahmi 1978; Olsson et al. 2010). *G. intraradices* modulated the production of spores directly to C availability, showing direct investment in reproduction as expected for r-strategists. In contrast, spore production of *D. reticulata* was not affected after a single defoliation and thus showed higher resistance to fluctuating C levels, as expected for K-strategists (Ijdo et al. 2010). Recent work has shown that plants supply more carbohydrates to fungal partners that provide more phosphorus and *vice versa* (Hammer et al. 2011; Kiers et al. 2011; Fellbaum et al. 2012, 2014; Bucking et al. 2016) giving rise to the 'fair trade' in 'biological market theory' (Fig. 9.3a). Kiers et al. (2011) used the model plant *Medicago truncatula* and three AM fungal species (*Glomus intraradices*, *G. custos* and *G. aggregatum*) to demonstrate this theory to explain the mutualism in AM symbiosis. These AM fungi exhibited either high or low levels of cooperation (symbiont quality), based on plant growth responses, costs of carbon per unit P transferred and resource-hoarding strategies. According to these traits, two species were classified as less-cooperative species directing more carbon resources either into storage vesicles—*G. aggregatum* or spores—*G. custos*, while *G. intraradices* was termed the cooperative species. Although colonization with all single species inoculation was above 80%, in two-species and three-species experiments, the cooperative fungus, *G. intraradices*, was significantly more enriched with host ¹³C than both less-cooperative species of the same genus. The cooperative species also transferred more P to roots with greater access to C resources, confirming that fungi can discriminate among hosts differing in C supply. In contrast, the less-cooperative species, *G. aggregatum*, responded differently. Like the cooperative species, it transferred more P to the root compartment with access to more C, showing that it was able to assess and respond to the rate of C supply. However, this species predominantly stored the P resources in long-chained polyphosphates, a host-inaccessible form which potentially reduces P availability for competing fungi and P directly available for host uptake. The investigations thus illustrate key differences in fungal strategies, with *G. intraradices* being a 'reciprocator' and *G. aggregatum* a less cooperative 'hoarder'. Mensah et al. (2015) have also suggested that the high functional diversity within species of AM fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. Recently, Walder and van der Heijden (2015) challenged the importance of reciprocally regulated exchange and thereby market dynamics, for resource exchange in the AM symbiosis, and suggested that such reciprocity is only found in

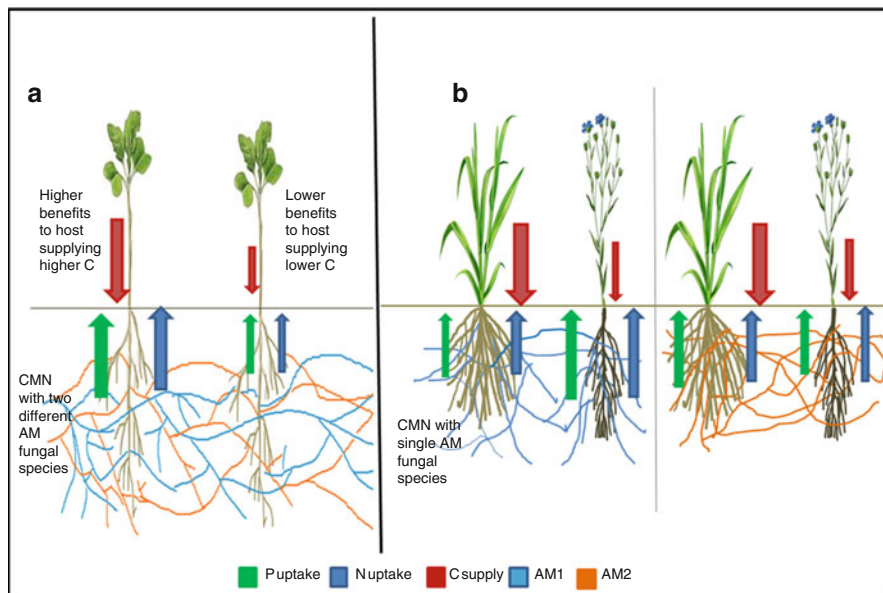


Fig. 9.3 Two different approaches to explain the nutrient exchange and functional diversity in AM symbioses: (a) ‘fair trade’ in ‘biological market theory’ (Kiers et al. 2011) where different AM fungi discriminate among hosts and transfer more nutrients to hosts providing higher C supply and (b) ‘unequal terms of trade’ (Walder et al. 2012; Walder and van der Heijden 2015) wherein the symbiosis between plants and AM fungi is not so tightly controlled and instead is based on exchange of ‘luxury goods’

a subset of symbionts, under specific conditions. Instead they proposed that it could be the exchange of luxury goods and sink strength that controls resource exchange in the plant—AM fungal symbiosis (Fig. 9.3). To study this, they set up microcosms containing sorghum and flax plants, interlinked by a common mycorrhizal network (CMN) of *Glomus intraradices* or *Glomus mosseae* and assessed the carbon investment of the two plants into the CMN through stable isotope tracing (Walder et al. 2012; Arguello et al. 2016). The plants’ ‘return of investment’ (i.e. the acquisition of nutrients via CMN) using ^{15}N and ^{33}P as tracers was also calculated. They observed that nutritional benefit to the two host plants strongly depended on the fungus involved: in the case of *G. intraradices*, flax behaved as a ‘cheater’ on sorghum, acquiring 80% to 90% of the total labeled nitrogen and phosphorus provided by the CMN, whereas the acquisition of labelled nitrogen and phosphorus was more balanced in the case of *G. mosseae*. In mixed cultures containing both AM fungi, sorghum, in return for a similar expenditure of carbon, received much more phosphorus from *G. mosseae* than from *G. intraradices*, whereas for flax it was the inverse. This agrees with the theory that the symbiosis between plants and AM fungi is based on the exchange of ‘luxury goods’ (Kiers and van der Heijden 2006) with the symbionts offering luxury goods in exchange for more limited resources (Fig. 9.3b). Therefore, Walder et al. 2012 argue that the biological

trade is not simply reciprocal or ‘fair exchange’ as proposed by Kiers et al. (2011), rather it depends on transient sink strengths and the efficiency of exchanges at the various symbiotic interfaces which may differ for different plant—AM fungal combinations. However, the debate between the two groups regarding the nutrient exchange in the underground market continues with both groups citing evidences in their favour (Kiers et al. 2016; van der Heijden and Walder 2016).

9.6 Conclusion

AM fungi are one of the most abundant symbionts prevalent in the world ecosystems. However, this mycorrhizal association is not a homogeneous association; each association of plant and fungus species combination depends strongly on the particular partners involved. Each AM is essentially a phenotypic response to the different fungal and plant genotypes involved and the environment they inhabit. The AM fungi may vary in germination patterns, in hyphal traits, in nutrient uptake and transfer capacity as well as in symbiotic efficiency. This functional diversity is also often found to be reflected in the gene expression patterns. The functional diversity of AM likely results from the genetic structure of AM fungi, which is multi-genomic and composed of hundreds or thousands of nuclei with different genetic composition. The use of molecular studies has indicated high genetic diversity within a population and even within a single spore. This genetic variation of nuclei in a single spore affects genetic diversity at the population level and plays a major role in increasing the functional diversity of AM fungi in ecosystems. Thus, in order to better understand the functional diversity of AM, it is imperative to study the pattern of genetic variation in AM fungi. Not only genetic diversity, different life strategies employed by different AM fungi are also directly responsible for the functional diversity observed. In order to get a clear understanding on functional diversity and the factors controlling it, further studies on the variable responses of AM fungi under controlled conditions need to be studied along with genetic variation studies to correlate the two.

Acknowledgements The authors are grateful to the Department of Biotechnology (DBT), Government of India for providing financial assistance for undertaking the research in the above context.

References

- Abbott LK, Robson AD (1985) Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytol* 99:245–255
- Abbott LK, Robson AD, Gazey C (1994) Selection of inoculants vesicular-arbuscular mycorrhizal fungi. In: Norris JR, Read D, Varma AK (eds) *Techniques for mycorrhizal research*. Academic, San Diego, pp 1–22

- Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. *Plant Physiol* 149:549–560
- Angelard C, Sanders IR (2011) Effect of segregation and genetic exchange on arbuscular mycorrhizal fungi in colonization of roots. *New Phytol* 189:652–657
- Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR (2010) Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription. *Curr Biol* 20:1216–1221
- Antunes PM, Koch AM, Morton JB, Rillig MC, Klironomos JN (2011) Evidence for functional divergence in arbuscular mycorrhizal fungi from contrasting climatic origins. *New Phytol* 189:507–514
- Arguello A, O'Brien MJ, van der Heijden MGA, Wiemken A, Schmid B, Niklaus PA (2016) Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. *Ecol Lett* 19(6):648–656. doi:[10.1111/ele.12601](https://doi.org/10.1111/ele.12601)
- Arnheim N, Krystal M, Schmickel R, Wilson G, Ryder O, Zimmer E (1980) Molecular evidence for genetic exchanges among ribosomal genes on non-homologous chromosomes in man and apes. *Proc Natl Acad Sci U S A* 77:7323–7327
- Augé RM, Toler HD, Saxton AM (2014) Arbuscular mycorrhizal symbiosis and osmotic adjustment in response to NaCl stress: a meta-analysis. *Front Plant Sci* 5:562. doi:[10.3389/fpls.2014.00562](https://doi.org/10.3389/fpls.2014.00562)
- Avio L, Pellegrino E, Bonari E, Giovannetti M (2006) Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks. *New Phytol* 172:347–357
- Avise J (2004) Molecular markers, natural history, and evolution, 2nd edn. Sinauer, Sunderland, MA
- Bago B, Azcon-Aguilar C, Goulet A, Piché Y (1998) Branched absorbing structures (BAS): a feature of the extraradical mycelium of symbiotic arbuscular mycorrhizal fungi. *New Phytol* 139:375–388
- Balzergue C, Chabaud M, Barker DG, Bécard G, Rochange SF (2013) High phosphate reduces host ability to develop arbuscular mycorrhizal symbiosis without affecting root calcium spiking responses to the fungus. *Plant Nutr* 4:426. doi:[10.3389/fpls.2013.00426](https://doi.org/10.3389/fpls.2013.00426)
- Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE (1998) A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: Isolation and preliminary characterisation. *Plant J* 15:791–797
- Bécard G, Pfeffer PE (1993) Status of nuclear division in arbuscular mycorrhizal fungi during in vitro development. *Protoplasma* 174:62–68
- Begon, M., Harper, J.L., Townsend, C.R. (1996) Ecology: individuals, populations and communities. Blackwell Science, Oxford
- Bever JD, Wang M (2005) Arbuscular mycorrhizal fungi-hyphal fusion and multigenomic structure. *Nature* 433:3–4
- Bever JD, Kang HJ, Kaonongbua W, Wang M (2008) Genomic organization and mechanisms of inheritance in arbuscular mycorrhizal fungi: contrasting the evidence and implications of current theories. In: Varma A (ed) *Mycorrhiza*. Springer, Berlin, Heidelberg, pp 135–171
- Biermann B, Linderman RG (1983) Mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. *New Phytol* 95:97–105
- Boddington CL, Dodd JC (1999) Evidence that differences in phosphate metabolism in mycorrhizas formed by species of *Glomus* and *Gigaspora* might be related to their life-cycle strategies. *New Phytol* 142:531–538
- Bonfante P, Requena N (2011) Dating in the dark: How roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis. *Curr Opin Plant Biol* 14:451–457
- Boon E, Zimmerman E, Lang BF, Hijri M (2010) Intra-isolate genome variation in arbuscular mycorrhizal fungi persists in the transcriptome. *J Evol Biol* 23:1519–1527
- Boon E, Zimmerman E, St-Arnaud M, Hijri M (2013) Allelic differences among sister spores suggest genetic drift in an arbuscular mycorrhizal fungus. *PLoS One* 8:e83301. doi:[10.1371/journal.pone.0083301](https://doi.org/10.1371/journal.pone.0083301)

- Boon E, Halary S, Baptiste E, Hijri M (2015) Studying genome heterogeneity within the arbuscular mycorrhizal fungal cytoplasm. *Genome Biol Evol* 7:505–521
- Börstler B, Raab PA, Thiéry O, Morton JB, Redecker D (2008) Genetic diversity of the arbuscular mycorrhizal fungus *Glomus intraradices* as determined by mitochondrial large subunit rRNA gene sequences is considerably higher than previously expected. *New Phytol* 180:452–465
- Breuillin F, Schramm J, Hajirezaei M, Ahkami A, Favre P, Druege U et al (2010) Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. *Plant J* 64:1002–1017. doi:10.1111/j.1365-313X.2010.04385.x
- Brundrett MC (2004) Diversity and classification of mycorrhizal associations. *Bot Rev* 79:473–495
- Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320:37–77
- Bucking H, Liepold E, Ambilwade P (2012) The role of the mycorrhizal symbiosis in nutrient uptake of plants and the regulatory mechanisms underlying these transport processes. In: Dhal NK, Sahu SC (eds) *Plant science*. Intech, Janeza Trdine, pp 107–539
- Bucking H, Mensah JA, Fellbaum CA (2016) Common mycorrhizal networks and their effect on the bargaining power of the fungal partner in the arbuscular mycorrhizal symbiosis. *Commun Integr Biol* 9(1):e1107684. (4 pages). doi:10.1080/19420889.2015
- Burleigh SH, Cavagnaro T, Jakobsen I (2002) Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in Plant nutr. *J Exp Bot* 53:1593–1601
- Calvente R, Cano C, Ferrol N, Azcón-Aguilar C, Barea JM (2004) Analysing natural diversity of arbuscular mycorrhizal fungi in olive tree (*Olea europaea* L.) plantations and assessment of the effectiveness of native fungal isolates as inoculants for commercial cultivars of olive plantlets. *Appl Soil Ecol* 26:11–19
- Caravaca F, Alguacil MM, Figueroa D, Barea JM, Roldán A (2003) Re-establishment of *Retama sphaerocarpa* as a target species for reclamation of soil physical and biological properties in a semiarid Mediterranean land. *For Ecol Manag* 182:49–58
- Carbonnel S, Gutjahr C (2014) Control of arbuscular mycorrhiza development by nutrient signals. *Front Plant Sci* 5:Art 462
- Cavagnaro TR, Gao LL, Smith SE (2001) Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytol* 151:469–475
- Chiou TJ, Liu H, Harrison MJ (2001) The spatial expression patterns of a phosphate transporter (MtPT1) from *Medicago truncatula* indicate a role in phosphate transport at the root/soil interface. *Plant J* 25:281–293
- Clapp JP, Fitter AH, Young JPW (1999) Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. *Mol Ecol* 8:915–921
- Clapp JP, Rodriguez A, Dodd JC (2001) Inter- and intra-isolate rRNA large subunit variation in *Glomus coronatum* spores. *New Phytol* 149:539–554
- Colard A, Angelard C, Sanders IR (2011) Genetic exchange in an arbuscular mycorrhizal fungus results in increased rice growth and altered mycorrhiza-specific gene transcription. *Appl Environ Microbiol* 77:6510–6515
- Cooke JC, Gemma JN, Koske RE (1987) Observations of nuclei in vesicular-arbuscular mycorrhizal fungi. *Mycologia* 79:331–333
- Corradi N, Kuhn G, Sanders IR (2004) Monophyly of b-tubulin and H⁺-ATPase gene variants in *Glomus intraradices*: consequences for molecular evolutionary studies of AM fungal genes. *Fungal Genet Biol* 41:262–273
- Corradi N, Croll D, Colard A, Kuhn G, Ehinger M, Sanders IR (2007) Gene copy number polymorphisms in an arbuscular mycorrhizal fungal population. *Appl Environ Microbiol* 73:366–369
- Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N, Sanders IR (2008) Genetic diversity and host plant preferences revealed by simple sequence repeat and mitochondrial

- markers in a population of the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* 178:672–687
- Croll D, Giovannetti M, Koch AM, Sbrana C, Ehinger M, Lammers PJ, Sanders IR (2009) Nonsel­f vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* 181:924–937
- Daft MJ, El Giahmi AA (1978) Effects of arbuscular mycorrhiza on plant growth. VIII. Effects of defoliation and light on selected hosts. *New Phytol* 80:365–372
- de la Providencia IE, de Souza FA, Fernandez F, Sejalon-Delmas N, Declerck S (2005) Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenetic groups. *New Phytol* 165:261–271
- de Souza FA, Dalpé Y, Declerck S, de la Providencia IE, Sejalon-Delmas N (2005) Life history strategies in Gigasporaceae: insight from monoxenic culture. In: Declerck S, Strullu DG, Fortin JA (eds) *In vitro* culture of mycorrhizas. Springer, Heidelberg, pp 73–91
- Dickson S (2004) The Arum-Paris continuum of mycorrhizal symbioses. *New Phytol* 163:187–200
- Dickson S, Smith FA, Smith SE (2007) Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza* 17:375–393
- Douds DD, Nagahashi G (2000) Signalling and recognition events prior to colonisation of roots by arbuscular mycorrhizal fungi. In: Podila G, Douds DD (eds) *Current advances in mycorrhizae research*. APS Press, Minnesota, pp 11–18
- Dover G (1982) Molecular drive: a cohesive mode of species evolution. *Nature* 299:111–117
- Drew EA, Murray RS, Smith SE, Jakobsen I (2003) Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant Soil* 251:105–114
- Drigo B, Pijl AS, Duyts H, Kielak AM, Gamper HA, Houtekamer MJ, Boschker HTS, Bodelier PLE, Whiteley AS, Veen JAV, Kowalchuk GA (2010) Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO₂. *Proc Natl Acad Sci U S A* 107:10938–10942
- Ehinger MO, Croll D, Koch AM, Sanders IR (2012) Significant genetic and phenotypic changes arising from clonal growth of a single spore of an arbuscular mycorrhizal fungus over multiple generations. *New Phytol* 196:853–861
- Estrada B, Barea JM, Aroca R, Ruiz-Lozano JM (2013a) A native *Glomus intraradices* strain from a Mediterranean saline area exhibits salt tolerance and enhanced symbiotic efficiency with maize plants under salt stress conditions. *Plant Soil* 366:333–349
- Estrada B, Aroca R, Maathuis FJM, Barea JM, Ruiz-Lozano JM (2013b) Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. *Plant Cell Environ* 36:1771–1782
- Feddermann N, Boller T, Salzer P, Elfstrand S, Wiemken A, Elfstrand M (2008) *Medicago truncatula* shows distinct patterns of mycorrhiza-related gene expression after inoculation with three different arbuscular mycorrhizal fungi. *Planta* 227:671–680
- Feddermann N, Finlay R, Boller T, Elfstrand M (2010) Functional diversity in arbuscular mycorrhiza—the role of gene expression, phosphorous nutrition and symbiotic efficiency. *Fungal Ecol* 3:1–8
- Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bucking H (2012) Carbon availability triggers fungal nitrogen uptake and transport in the arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci U S A* 109:2666–2671
- Fellbaum CR, Mensah JA, Cloos AJ, Strahan GE, Pfeffer PE, Kiers ET, Bucking H (2014) Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytol* 203:646–656
- Friese C, Allen MF (1991) The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. *Mycologia* 83:409–418
- Gallaud I (1905) E'tudes sur les mycorrhizes endotrophes. *Rev Ge'n Bot* 17:5–48, 66–83, 123–135, 223–239, 313–325, 425–433, 479–500

- Gao LL, Knogge W, Delp G, Smith FA, Smith SE (2004) Expression patterns of defense-related genes in different types of arbuscular mycorrhizal development in wild-type and mycorrhiza defective mutant tomato. *Mol Plant-Microbe Interact* 17:1103–1113
- Garg N, Pandey R (2015) Effectiveness of native and exotic arbuscular mycorrhizal fungi on nutrient uptake and ion homeostasis in salt-stressed *Cajanus cajan* L. (Millsp.) genotypes. *Mycorrhiza* 25:165–180
- Garg N, Pandey R (2016) High effectiveness of exotic arbuscular mycorrhizal fungi is reflected in improved rhizobial symbiosis and trehalose turnover in *Cajanus cajan* genotypes grown under salinity stress. *Fungal Ecol* 21:57–67
- Geneva M, Zehirov G, Djonova E, Kaloyanova N, Georgiev G, Stancheva I (2006) The effect of inoculation of pea plants with mycorrhizal fungi and Rhizobium on nitrogen and phosphorus assimilation. *Plant Soil Environ* 52:435–440
- Genre A, Chabaud M, Faccio A, Barker DG, Bonfante P (2008) Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *Plant Cell* 20:1407–1420
- Gianinazzi-Pearson V, Van Tuinen D, Wipf D, Dumas-Gaudot E, Recorbet G, Liu Y, Doidy J, Redecker D, Ferrol N (2012) Exploring the genome of glomeromycotan fungi. In: Hock B (ed) *Fungal associations*. Springer, Berlin, Heidelberg, pp 1–21
- Giovannetti M, Fortuna P, Citernesi AS, Morini S, Nuti MP (2001) The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. *New Phytol* 151:717–724
- Giovannetti M, Sbrana C, Strani P, Agnolucci M, Rinaudo V, Avio L (2003) Genetic diversity of isolates of *Glomus mosseae* from different geographic areas detected by vegetative compatibility testing and biochemical and molecular analysis. *Appl Environ Microbiol* 69:616–624
- Giovannetti M, Sbrana C, Avio L, Strani P (2004) Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. *New Phytol* 164:175–181
- Giri B, Giang PH, Kumari R, Prasad R, Sachdev M, Garg AP, Oelmüller R, Varma A (2005) Mycorrhizosphere: strategies and functions. In: Buscot F, Varma A (eds) *Microorganisms in soils: roles in genesis and functions*, vol 3. Springer, Berlin, pp 213–252
- Gollotte A, Van Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza* 14:111–117
- Gollotte A, L'Haridon F, Chatagnier O et al (2006) Repetitive DNA sequences include retrotransposons in genomes of the Glomeromycota. *Genetica* 128:455–469
- Grace EJ, Cotsaftis O, Tester M, Smith FA, Smith SE (2009) Arbuscular mycorrhizal inhibition of growth in barley cannot be attributed to extent of colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. *New Phytol* 181:938–949
- Grunwald U, Guo WB, Fischer K, Isayenkov S, Ludwig-Müller J, Hause B, Yan XL, Kuster H, Franken P (2009) Overlapping expression patterns and differential transcript levels of phosphate transporter genes in arbuscular mycorrhizal, Pi-fertilised and phytohormone-treated *Medicago truncatula* roots. *Planta* 229:1023–1034
- Gustafson DJ, Casper BB (2006) Differential host plant performance as a function of soil arbuscular mycorrhizal fungal communities: experimentally manipulating co-occurring *Glomus* species. *Plant Ecol* 183:257–263
- Gutjahr C, Parniske M (2013) Cell and developmental biology of the arbuscular mycorrhiza symbiosis. *Annu Rev Cell Dev Biol* 29:593–617
- Gutjahr C, Banba M, Croset V, An K, Miyao A, An G, Hirochika H, Imaizumi-Anraku H, Paszkowski U (2008) Arbuscular mycorrhiza-specific signaling in rice transcends the common symbiosis signaling pathway. *Plant Cell* 20:2989–3005
- Hamby RK, Zimmer EA (1992) Ribosomal RNA as a phylogenetic tool in plant systematics. In: Soltis PS, Soltis DE, Doyle JJ (eds) *Molecular systematics of plants*. Chapman and Hall, New York, pp 50–91

- Hamel C, Fyles H, Smith DL (1990) Measurement of development of endomycorrhizal mycelium using three vital stains. *New Phytol* 115:297–302
- Hammer EC, Pallon J, Wallander H, Olsson PA (2011) Tit for Tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiol Ecol* 76:236–244
- Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14:2413–2429
- Hart MM, Reader RJ (2002) Host plant benefit from association with arbuscular mycorrhizal fungi: variation due to differences in size of mycelium. *Biol Fertil Soils* 36:357–366
- Hart MM, Reader RJ, Klironomos JN (2001) Life-history strategies of arbuscular mycorrhizal fungi in relation to their successional dynamics. *Mycologia* 93:1186–1194
- Herrera Medina MJ, Gagnon H, Piche Y, Ocampo JA, Garrido JMG, Vierheilig H (2003) Root colonization by arbuscular mycorrhizal fungi is affected by the salicylic acid content of the plant. *Plant Sci* 164:993–998
- Hijri M, Sanders IR (2004) The arbuscular mycorrhizal fungus *Glomus intraradices* is haploid and has a small genome size in the lower limit of eukaryotes. *Fungal Genet Biol* 41:253–261
- Hijri M, Sanders IR (2005) Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature* 433:160–163
- Hijri M, Hosny M, van Tuinen D, Dulieu H (1999) Intraspecific ITS polymorphism in *Scutellospora castanea* (Glomales, Zygomycota) is structured within multinucleate spores. *Fungal Genet Biol* 26:141–151
- Hijri M, Redecker D, Petetot JAMC, Voigt K, Wostemeyer J, Sanders IR (2002) Identification and isolation of two ascomycete fungi from spores of the arbuscular mycorrhizal fungus *Scutellospora castanea*. *Appl Environ Microbiol* 68:4567–4573
- Hohnjec N, Vieweg MF, Pühler A, Becker A, Küster H (2005) Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza. *Plant Physiol* 137:1283–1301
- Hosny M, deBarros JPP, Gianinazzi-Pearson V, Dulieu H (1997) Base composition of DNA from glomalean fungi: high amounts of methylated cytosine. *Fungal Genet Biol* 22:103–111
- Hosny M, Gianinazzi-Pearson V, Dulieu H (1998) Nuclear DNA content of 11 fungal species in glomales. *Genome* 41:422–428
- Huang JC, Lai WA, Singh S, Hameed A, Young CC (2013) Response of mycorrhizal hybrid tomato cultivars under saline stress. *J Soil Sci Plant Nutr* 13:469–484
- IJdo M, Schtickzelle N, Cranenbrouck S, Declerck S (2010) Do arbuscular mycorrhizal fungi with contrasting life-history strategies differ in their responses to repeated defoliation? *FEMS Microbiol Ecol* 72:114–122
- Jakobsen I (1999) Transport of phosphorus and carbon in arbuscular mycorrhizas. In: Varma A, Hock B (eds) *Mycorrhiza: structure, function, molecular biology and biotechnology*. Springer, Berlin, pp 309–332
- Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I. spread of hyphae and phosphorus inflow into roots. *New Phytol* 120:371–380
- Jansa J, Mozafar A, Frossard E (2003) Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. *Agronomie* 23:481–488
- Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytol* 177:779–789. doi:10.1111/j.1469-8137.2007.02294.x
- Jany JL, Pawlowska TE (2010) Multinucleate spores contribute to evolutionary longevity of asexual glomeromycota. *Am Nat* 175:424–435
- Javot H, Pumplin N, Harrison M (2007) Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant Cell Environ* 30:310–322

- Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol* 135:575–585
- Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton LM, Allen EB (2003) Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology* 84:1895–1908
- Karandashov V, Nagy R, Wegmuller S, Amrhein N, Bucher M (2004) Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci U S A* 101:6285–6290
- Kiers ET, van der Heijden MGA (2006) Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology* 87:1627–1636
- Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuysse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333:880–882
- Kiers ET, West SA, Wyatt GAK, Gardner A, Bücking H, and Werner GDA (2016) Misconceptions on the application of biological market theory to the mycorrhizal symbiosis. *Nat Plants* 2:16063. doi:[10.1038/nplants.2016.63](https://doi.org/10.1038/nplants.2016.63)
- Kivlin SN, Hawkes CV, Treseder KK (2011) Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biol Biochem* 43:2294–2303
- Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84:2292–2301
- Klironomos JN, Hart MM (2002) Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza* 12:181–184
- Koch AM, Kuhn G, Fontanillas P, Fumagalli L, Goudet J, Sanders IR (2004) High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *Proc Natl Acad Sci U S A* 101:2369–2374
- Koch AM, Croll D, Sanders IR (2006) Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. *Ecol Lett* 9:103–110
- Kohl L, van der Heijden MGA (2016) Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. *Soil Biol Biochem* 94:191–199
- Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM et al (2013) Towards a unified paradigm for sequence-based identification of fungi. *Mol Ecol* 22:5271–5277
- Kuhn G, Hijri M, Sanders IR (2001) Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* 414:745–748
- Lambert DH, Cloe H, Baker DE (1980) Variation in the response of Alfa alfa clones and cultivars of mycorrhiza and phosphorus. *Crop Sci* 20:615–618
- Lee EH, Eo JK, Ka KH, Eom AH (2015) Diversity of Arbuscular mycorrhizal fungi and their roles in ecosystems. *Mycobiology* 41:121–125. doi:[10.5941/MYCO.2013.41.3.121](https://doi.org/10.5941/MYCO.2013.41.3.121)
- Lerat S, Lapointe L, Gutjahr S, Piché Y, Vierheilig H (2003a) Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent. *New Phytol* 157:589–595
- Lerat S, Lapointe L, Piche Y, Vierheilig H (2003b) Variable carbon sink strength of different *Glomus mosseae* strains colonizing barley roots. *Can J Bot* 81:886–889
- Li H, Smith FA, Dickson S, Holloway RE, Smith SE (2008) Plant growth depressions in arbuscular mycorrhizal symbioses: not just caused by carbon drain? *New Phytol* 178:852–862
- Lin K, Limpens E, Zhang Z, Ivanov S, Saunders DGO, Mu D, Pang E, Cao H, Cha H, Lin T, Zhou Q, Shang Y, Li Y, Sharma Y, van Velzen R, de Ruijter N, Aanen DK, Win J, Kamoun S, Bisseling T, Geurts R, Huanget S (2014) Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS Genet* 10:e1004078. doi:[10.1371/journal.pgen.1004078](https://doi.org/10.1371/journal.pgen.1004078)

- Liu J, Blaylock L, Endre G, Cho J, Town CD (2003) Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of the arbuscular mycorrhizal symbiosis. *Plant Cell* 15:2106–2123
- Maherali H, Klironomos JN (2012) Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. *PLoS One* 7:e36695. doi:[10.1371/journal.pone.0036695](https://doi.org/10.1371/journal.pone.0036695)
- Marleau J, Dalpe Y, St-Arnaud M, Hijri M (2011) Spore development and nuclear inheritance in arbuscular mycorrhizal fungi. *BMC Evol Biol* 11:51. doi:[10.1186/1471-2148-11-51](https://doi.org/10.1186/1471-2148-11-51)
- Marulanda A, Azcón R, Ruiz-Lozano JM (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. *Physiol Plant* 119:526–533
- Meghvansi MK, Prasad K, Harwani D, Mahna SK (2008) Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. *Eur J Soil Biol* 44:316–323
- Mensah JA, Koch AM, Antunes PM, Kiers ET, Hart M, Bücking H (2015) High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. *Mycorrhiza*. doi:[10.1007/s00572-015-0631-x](https://doi.org/10.1007/s00572-015-0631-x)
- Munkvold L, Kjølner R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytol* 164:357–364
- Nadal M, Paszkowski U (2013) Polyphony in the rhizosphere: Presymbiotic communication in arbuscular mycorrhizal symbiosis. *Curr Opin Plant Biol* 16:473–479
- Nouri E, Breuillin-Sessoms F, Feller U, Reinhardt D (2014) Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in *Petunia hybrida*. *PLoS One* 9:e90841. doi:[10.1371/journal.pone.0090841](https://doi.org/10.1371/journal.pone.0090841)
- Novais CB, Borges WL, Jesus ED, Saggini OJ, Siqueira JO (2014) Inter- and intraspecific functional variability of tropical arbuscular mycorrhizal fungi isolates colonizing corn plants. *Appl Soil Ecol* 76:78–86
- Olsson PA, Rahm J, Aliasgharzad N (2010) Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiol Ecol* 72:125–131
- Opik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I, Jairus T, Kalwij JM, Koorem K, Leal ME, Liira J, Metsis M, Neshataeva V, Paal J, Phosri C, Põlme S, Reier Ü, Saks Ü, Schimann H, Thiéry O, Vasar M, Moora M (2013) Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza* 23:411–430
- Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* 6:763–775
- Paszkowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci U S A* 99:13324–13329
- Pawlowska TE (2005) Genetic processes in arbuscular mycorrhizal fungi. *FEMS Microbiol Lett* 251:185–192
- Pawlowska TE (2007) How the genome is organized in the Glomeromycota. In: Heitman JW, Kronstad JW, Taylor JW, Casselton LA (eds) *Sex in fungi: molecular determination and evolutionary implications*. American Society for Microbiology, Washington, DC, pp 419–430
- Pawlowska TE, Taylor JW (2004) Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* 427:733–737
- Pawlowska TE, Taylor JW (2005) Arbuscular mycorrhizal fungi-hyphal fusion and multigenomic structure. *Reply*. *Nature* 433:4–5
- Pellegrino E, Bedini S (2014) Enhancing ecosystem services in sustainable agriculture: biofertilization and biofortification of chickpea (*Cicer arietinum* L.) by arbuscular mycorrhizal fungi. *Soil Biol. Biochem* 68:429–439
- Pellegrino E, Bedini S, Avio L, Bonari E, Giovannetti M (2011) Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. *Soil Biol Biochem* 43:367–376

- Peng J, Li Y, Shi P, Chen X, Lin H, Zhao B (2011) The differential behavior of arbuscular mycorrhizal fungi in interaction with *Astragalus sinicus* L. under salt stress. *Mycorrhiza* 21:27–33
- Pianka ER (1970) On r and K selection. *Am Nat* 104:592–597
- Poulsen KH, Nagy R, Gao LL, Smith SE, Bucher M, Smith FA, Jakobsen I (2005) Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. *New Phytol* 168:445–454
- Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *Proc R Soc B Biol Sci* 276:4237–4245
- Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: Historical development. In: Varma A, Prasad R, Tuteja N (eds) *Mycorrhiza*. Springer, Switzerland, pp 1–7
- Pringle A, Moncalvo JM, Vilgalys R (2000) High levels of variation in ribosomal DNA sequences within and among spores of a natural population of the arbuscular mycorrhizal fungus *Acaulospora colossica*. *Mycologia* 92:259–268
- Pringle A, Moncalvo JM, Vilgalys R (2003) Revisiting the rDNA sequence diversity of a natural population of the arbuscular mycorrhizal fungus *Acaulospora colossica*. *Mycorrhiza* 13:227–231
- Purin S, Morton JB (2011) In situ analysis of anastomosis in representative genera of arbuscular mycorrhizal fungi. *Mycorrhiza* 21:505–514
- Requena N, Perez-Solis E, Azcón-Aguilar C, Jeffries P, Barea JM (2001) Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Appl Environ Microbiol* 67:495–498
- Requena N, Serrano E, Ocon A, Breuninger M (2007) Plant signals and fungal perception during arbuscular mycorrhiza establishment. *Phytochemistry* 68:33–40
- Ruiz-Lozano JM, Azcon R (1993) Specificity and functional compatibility of VA mycorrhizal endophytes in association with *Bradyrhizobium* strains in *Cicer arietinum*. *Symbiosis* 15:217–226
- Schreiner RP (2007) Effects of native and nonnative arbuscular mycorrhizal fungi on growth and nutrient uptake of ‘Pinot noir’ (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus. *Appl Soil Ecol* 36:205–215
- Schüßler A (2014) Glomeromycota: Species list. <http://schuessler.userweb.mwn.de/amphylo> [accessed 9 November 2013]
- Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: Phylogeny and evolution. *Mycol Res* 105:1413–1421
- Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecol Lett* 9:501–515
- Sharma D, Kapoor R, Bhatnagar AK (2009) Differential growth response of *Curculigo orchioides* to native arbuscular mycorrhizal fungal (AMF) communities varying in number and fungal components. *Eur J Soil Biol* 45:328–333
- Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*. Academic Press, London
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*, 3rd edn. Academic Press, New York
- Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annu Rev Plant Biol* 62:227–250
- Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* 104:1–13
- Smith FA, Jakobsen I, Smith SE (2000) Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytol* 147:357–366
- Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol* 133:16–20

- Smith SE, Smith FA, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: The contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth and total P uptake. *New Phytol* 162:511–524
- Song F, Kong X, Dong A, Liu X (2012) Impact of arbuscular mycorrhizal fungi on the growth and related physiological indexes of *Amorpha fruticosa*. *J Med Plant Res* 6:3648–3655
- Stancheva I, Geneva M, Zehirov G, Tsvetkova G, Hristozkova M, Georgiev G (2006) Effects of combined inoculation of pea plants with arbuscular mycorrhizal fungi and *Rhizobium* on nodule formation and nitrogen fixing activity. *Gen Appl Plant Physiol Special Issue* 4:61–66
- Stukenbrock EH, Rosendahl S (2005) Clonal diversity and population genetic structure of arbuscular mycorrhizal fungi (*Glomus* sp.) studied by multilocus genotyping of single spores. *Mol Ecol* 14:743–752
- Sykorova Z, Ineichen K, Wiemken A, Redecker D (2007) The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment. *Mycorrhiza* 18:1–14
- Tajini F, Drevon J (2012) Effect of arbuscular mycorrhizas on P use efficiency for growth and N₂ fixation in common bean (*Phaseolus vulgaris* L.) *Sci Res Essays* 7:1681–1689
- Tchabi A, Coyne D, Hountondji F, Llawouin L, Wiemken A, Oehl F (2010) Efficacy of indigenous arbuscular mycorrhizal fungi for promoting white yam (*Dioscorea rotundata*) growth in West Africa. *Appl Soil Ecol* 45:92–100
- Thonar C, Schnepf A, Frossard E, Roose T, Jansa J (2011) Traits related to differences in function among three arbuscular mycorrhizal fungi. *Plant Soil* 339:231–245
- Tian CY, Feng G, Li XL, Zhang FS (2004) Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Appl Soil Ecol* 26:143–148
- Tian H, Drijber RA, Xiaolin L, Miller DN, Wienhold BJ (2013) Arbuscular mycorrhizal fungi differ in their ability to regulate the expression of phosphate transporters in maize (*Zea mays* L.) *Mycorrhiza* 23:507–514
- Tisserant E, Kohler A, Dozolme-Seddas P, Balestrini R, Benabdellah K, Colard A, Croll D, Da Silva C, Gomez SK, Koul R, Ferrol N, Fiorilli V, Formey D, Franken P, Helber N, Hijri M, Lanfranco L, Lindquist E, Liu Y, Malbreil M, Morin E, Poulain J, Shapiro H, van Tuinen D, Waschke A, Azcón-Aguilar C, Bécard G, Bonfante P, Harrison MJ, Küster H, Lammers P, Paszkowski U, Requena N, Rensing SA, Roux C, Sanders IR, Shachar-Hill Y, Tuskan G, Young JP, Gianinazzi-Pearson V, Martin F (2012) The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytol* 193:755–769
- Tisserant E, Malbreil M, Kuoc A, Kohlera A, Symeonidou A, Balestrini R, Charron P, Duensing N, dit Frey NF, Gianinazzi-Pearson V, Gilbert LB, Handa Y, Herr JR, Hijri M, Koul R, Kawaguchi M, Krajinski F, Lammers PJ, Masclaux FG, Murat C, Morin E, Steve Ndikumana S, Pagni M, Petitpierre D, Requena N, Rosikiewicz P, Riley R, Saito K, Clemente HS, Shapiro H, van Tuinen D, Bécard G, Bonfante P, Paszkowski U, Shachar-Hill Y, Tuskan GA, Young JPW, Sanders IR, Henrissat B, Rensing SA, Grigoriev IV, Corradi N, Roux C, Martin F (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proc Natl Acad Sci U S A* 110:20117–20122
- Torreillas E, Alguacil MM, Roldán A (2012) Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. *Appl Environ Microbiol* 78:6180–6186
- Treseder KK (2013) The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant Soil* 371:1–13
- Tufenkci S, Demir S, Sensoy S, Ünsal H, Demirel E (2012) The effects of arbuscular mycorrhizal fungi on the seedling growth of four hybrid cucumber (*Cucumis sativus* L.) cultivars. *Turk J Agric For* 36:317–327

- van der Heijden MGA, Scheublin TR (2007) Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytol* 174:244–250
- van der Heijden MGA, Walder F (2016) Reply to ‘Misconceptions on the application of biological market theory to the mycorrhizal symbiosis’. *Nat Plants* 2:16062. doi:[10.1038/nplants.2016.62](https://doi.org/10.1038/nplants.2016.62)
- van der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72
- van der Heijden MGA, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol* 205:1406–1423. doi:[10.1111/nph.13288](https://doi.org/10.1111/nph.13288)
- Vandenkoornhuysen P, Leyval C (1998) SSU rDNA sequencing and PCR-fingerprinting reveal genetic variation within *Glomus mosseae*. *Mycologia* 90:791–797
- Vandenkoornhuysen P, Leyval C, Bonnin I (2001) High genetic diversity in AM fungi: evidence for recombination events. *Heredity* 87:243–253
- Verbruggen E, van der Heijden MGA, Weedon JT, Kowalchuk GA, Røling WFM (2012) Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. *Mol Ecol* 21:2341–2353
- Veresoglou SD, Shaw LJ, Sen R (2011) *Glomus intraradices* and *Gigaspora margarita* arbuscular mycorrhizal associations differentially affect nitrogen and potassium nutrition of *Plantago lanceolata* in a low fertility dune soil. *Plant Soil* 340:481–490
- Voets L, de la Providencia IE, Declerck S (2006) Glomeraceae and Gigasporaceae differ in their ability to form hyphal networks. *New Phytol* 172:185–188
- Wagg C, Jansa J, Stadler M, Schmid B, Van der Heijden MGA (2011) Mycorrhizal fungal identity and diversity relaxes plant – plant competition. *Ecology* 92:1303–1313
- Walder F, van der Heijden MGA (2015) Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. *Nat Plants* 1:15159. doi:[10.1038/nplants.2015.159](https://doi.org/10.1038/nplants.2015.159)
- Walder F, Niemann H, Natarajan M, Lehmann MF, Boller T, Wiemken A (2012) Mycorrhizal networks: common goods of plants shared under unequal terms of trade. *Plant Physiol* 159:789–797
- Williams A, Ridgway H, Norton D (2013) Different arbuscular mycorrhizae and competition with an exotic grass affect the growth of *Podocarpus cunninghamii* Colenso cuttings. *New For* 44:183–195
- Wu QS, Li GH, Zou YN (2011) Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (*Prunus persica* L. Batsch) seedlings. *J Anim Plant Sci* 21:746–750
- Xu GH, Chague V, Melamed-Bessudo C, Kapulnik Y, Jain A, Raghobama KG, Levy AA, Silber A (2007) Functional characterization of LePT4: a phosphate transporter in tomato with mycorrhiza-enhanced expression. *J Exp Bot* 58:2491–2501
- Zhu YG, Miller RM (2003) Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends Plant Sci* 8:407–409

Chapter 10

Mycorrhizal Symbiosis: Ways Underlying Plant–Fungus Interactions

Shaily Javeria, Vivek Kumar, Pratibha Sharma, Lakshman Prasad, Manoj Kumar, and Ajit Varma

Abstract Dissimilar and diverse symbiotic mycorrhizal interactions within plants and fungi occur, which is almost ubiquitous and universal, in the broad range of global ecosystems. The entire mycorrhizal communications achieve symbiotic functioning through development of an extensive contact surface area between plant and fungal cells, where exchange of nutrients and signals takes place. The swap of beneficial molecules within the plant and the fungal cytoplasm takes place both through their cell walls and the plasma membranes, having a purposeful chamber, known as symbiotic interface. Amongst all symbiotic interfaces, the arbuscular mycorrhizal (AM) relationship has intricate intracellular interface which gains major consideration since its first portrayal. It is dissimilar in ectomycorrhizae (ECM); here the fungus grows outside and inside the roots cell walls, which are constantly in direct contact and form interface within both the partners. The mycorrhizae are diverse fungi belonging to dissimilar fungal taxa and interact with roots around of 90% plant species and supply important nutrients for their growth. This also hypothesizes the flow of energy-rich composites required for nutrient mobilization and simultaneously transportation of mobilized products back to their host. Traditionally, these have chiefly been considered within pretty precise perspective of their effects on devouring dissolved mineral nutrients by plants. Enormous research work has been done which put emphasis on multifarious outlook of the mycorrhizal association with plant and also with associated microbial communities and ultimately on ecosystem processes. Consequently, the inputs of both partners in mycorrhizal association are starting to be decrypted to understand this knowledge for enhanced and progressive agricultural practices. The foremost aim of this chapter is to understand the prevailed information on mycorrhizal communications and interactions by integrating morphological observations with plants.

S. Javeria • P. Sharma • L. Prasad
Biological Control Laboratory, Division of Plant Pathology, IARI, New Delhi, India

V. Kumar • M. Kumar • A. Varma (✉)
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Sector-125, Noida
201303, India
e-mail: ajitvarma@amity.edu

10.1 Introduction

Bernhardt Frank in 1885 coined the term “mycorrhiza” by identifying some special structures in tree roots. “Mycorrhiza” obtained from two Greek words, viz., myco means fungus and rhiza means root. Frank described its morphology as well as its physiological role (Strack and Fester 2006).

Mycorrhizas are found in many environments, and their ecological success reflects a high degree of diversity in the genetic and physiological abilities of the fungal endophytes (Bonfante and Anca 2009). About 6000 species in the Glomeromycotina, Ascomycotina, and Basidiomycotina have been recorded as mycorrhizal, and the advent of molecular techniques is increasing this number (Bonfante and Anca 2009).

H. Anton de Bary in 1869 described “Symbiosis” as a long-term closed interaction between two or more biologically distinct species, which may range from mutualism to parasitism. Later this term was used only with mutualistic association organisms, viz., lichens (Smith and Read 2008). Root colonizing fungi are associated with more than 90% of terrestrial plants, establishing stable and closer mutualistic symbiosis known as mycorrhiza, which generate a huge hyphal network in the soil, which also associate with complete plant communities offering nutrients and energy flow within soil and plants (Cardon and Whitbeck 2007; Prasad et al. 2017), while the association and the relationships of roots and fungi are known as mycorrhizal associations, which are taking part in the nutrients absorption from the soil, and mostly found within fungal hyphae and plant’s underground organs. This association is one of the most important associations in this planet (Mohammadi 2011). Mycorrhiza increases significantly in surface area of plant root by production of extensive hypha which enhances plant growth under relatively harsh conditions, viz., deficiency of nutrients and drought stress, etc. (Mohammadi 2011; Prasad et al. 2017).

10.1.1 *Mycorrhiza: Plant–Fungus Communication*

Classically, a mycorrhiza is defined as an interaction from which both partners benefit. Generally, it is claimed that mycorrhizal fungi improve plant nutrient uptake thanks to fine exploration of the rhizosphere by the hyphae, which in return receive plant carbohydrates that are essential for completion of the fungal life cycle. This retains the concept of mutualism, i.e., an interaction of net benefit to both parties (Thompson and Cunningham 2002) and poses questions about the molecular mechanisms that allow nutritional exchange. A breakthrough on this front has come from some important findings: AM fungi possess active phosphate transporters that take up inorganic phosphate (Pi) from the soil, allowing its delivery to the plant (Harrison and Buuren 1995). Furthermore, plants also possess phosphate transporters that are mycorrhiza specific. Their role is to receive Pi from the fungus and

deliver it to plant cells. A *Medicago truncatula* Pi transporter exclusively expressed during AM symbiosis and located in the periarbuscular membrane (Harrison 2005) not only is essential for acquisition of Pi delivered by the AM fungus but is also required to maintain arbuscule vitality and sustain development of the fungus (Javot et al. 2007). Pi transport therefore seems to be a signal to sustain fungal growth inside the root and a determinant of arbuscule morphogenesis. Nitrogen is the other important element taken up by most mycorrhizal fungi. Genes involved in organic and inorganic uptake of N have been identified in AM and ECM fungi (Cappellazzo et al. 2008; Lucic et al. 2008; Smith and Read 2008). Many molecular and physiological data show that plant N transporters are activated during mycorrhization (Guether et al. 2009a, b; Smith and Read 2008), suggesting that mycorrhizal fungi release a substantial amount of N to their hosts. While these fungal and plant transporters may be used as clear markers of mycorrhizal function, the reverse nutrient flow is not so clearly characterized. Carbon transfer from plants to mycorrhizal fungi was demonstrated in the 1960s (Smith and Read 2008), but the molecular mechanisms are still unclear. With the exception of the gene described in the glomeromycotan *Geosiphon pyriforme* (Schüßler et al. 2006), which forms symbiosis with a cyanobacterium, and of the *AmMstI* gene from the ECM fungus *Amanita muscaria* (Nehls et al. 1998), no other hexose transporter responsible for the uptake of C released by host cells has so far been characterized in mycorrhizal fungi. In addition, the transfer does not always go in the expected direction; for example, in orchid mycorrhizas or in other heterotrophic plants, C moves from the fungus toward the plant (Selosse and Roy 2009). In this case, the nature of the benefit for the fungus is not obvious, although it might gain advantages, for example, by living within a protected niche.

A crucial consequence of nutrient exchange is that the partners must be living and in physical contact through their cell surfaces (Bonfante 2001). The result is a specialized interface that is particularly complex during intracellular interactions. Here, the fungus is in all cases engulfed by a plant-derived membrane, one result of a developmental program leading to intracellular accommodation of microbes by plants (Parniske 2000). In AM, this new compartment is known as the interfacial compartment and consists of the invaginated host membrane, cell wall-like material, and the fungal wall and plasma membrane (Bonfante 2001). Cellular and molecular approaches have provided many insights into the structure, function, and biogenesis of this complex compartment (Guether et al. 2009a; Harrison 2005; Parniske 2000). Some fungi, such as *Piriformospora indica*, are sometimes defined as mycorrhizal because of their capacity to stimulate plant growth, even if an interface between living partners is not always present and the fungus may surprisingly cause the death of plant cells (Deshmukh et al. 2006).

10.1.2 Fundamental Assortment of Mycorrhizal Interactions

Arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) associations differ in their structural characteristics and in the plant and fungal species that they involve. In AM roots, the fungus penetrates intercellularly and intracellularly into the root cortex, whereas in ECM roots the fungus only penetrates intercellularly into the root cortex. The main structural differences between AM and ECM associations of angiosperms or gymnosperms are discussed (Fig. 10.1) (Bücking et al. 2012).

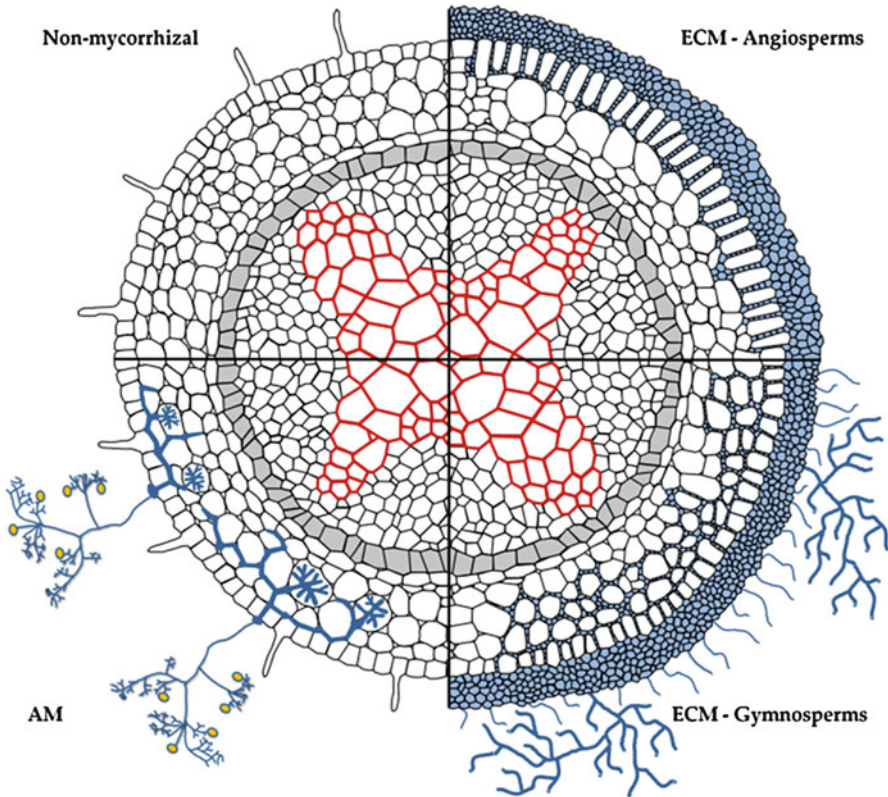


Fig. 10.1 Arbuscular mycorrhizal (AM) structural characteristics of gymnosperms or angiosperms of ectomycorrhizal (ECM) root adapted from Bücking et al. (2012)

10.2 Arbuscular Mycorrhizal Fungi Symbiosis

Endomycorrhizas are further divided into orchid, ericoid, and arbuscular mycorrhizas (Smith and Read 2008). There are numerous types of mycorrhizas present in nature, but endotrophic arbuscular mycorrhiza (AM) is the most common type, present in large number of plant species. Nearly all herbaceous plants, shrubs, and trees of temperate and tropical habitats can form arbuscular mycorrhizas. “Vesicular-arbuscular mycorrhiza” (VAM) was the earlier term replaced by “Arbuscular mycorrhiza” (AM) because all endomycorrhizas didn’t produce vesicles, but all produce arbuscules. The “arbuscular” as a name was derived from the distinct structure known as the arbuscules, which is present in the cortical cell of plants root (Smith and Read 2008). For the diagnostic purposes of AM symbioses, these structures are used.

The AM symbioses, formed between soil fungi and vascular plants, have a long history, with fossils providing evidence of AM fungi in the roots of the earliest land plants more than 400 m years (Remy et al. 1994). Sequence data and fossils of spores and hyphae point to the existence of AM fungi even earlier, more than 460 m years, and it is suggested that the AM fungi assisted plants in their colonization of land (Pirozynski and Malloch 1975; Redeker et al. 2000; Simon et al. 1993). Certainly, it is clear that the ability to form an AM symbiosis occurred early in the evolution of plants, and today the capacity to form these associations is distributed widely throughout the plant kingdom and includes angiosperms, gymnosperms, pteridophytes, and some bryophytes. More than 150 species of AM fungi belong to the Zygomycota which includes in the Glomales (Morton and Benny 1990).

Within the angiosperms, at least 80% of the species are able to form AM symbioses (Harley and Harley 1987; Newman and Reddell 1987; Smith and Read 1997) AM fungi are obligate symbionts that establish a symbiosis with the plant in order to obtain carbon, which enables them to grow and complete their life cycle. Their main contribution is to assist the plant with the acquisition of mineral nutrients, particularly phosphorus, and recently it was suggested that in an AM symbiosis, plants receive all of their phosphorus via their fungal symbiont (Smith et al. 2003). Phosphorus is an essential mineral nutrient that constitutes up to 0.2% (dry weight) of each plant cell and is thus required in significant quantities (Schachtman et al. 1998). In many soils, the concentration of phosphorus available to plants is limiting for growth (Holford 1997). Consequently, improvements in phosphorus acquisition have a significant impact on plant growth, health, and subsequently on plant biodiversity and ecosystem productivity (Smith and Read 1997). While enhanced plant mineral nutrition is of immense significance, other aspects of the AM symbiosis have far-reaching effects (Newsham et al. 1995). The extra radical phase of the arbuscular mycorrhiza includes meters of AM fungal hyphae that impact soil aggregate stability (Bearden and Petersen 2000; Requena et al. 2001; Rillig et al. 2003). Furthermore, AM fungi receive 100% of their carbon from the plant and this increase in carbon flow to the roots, estimated at up to 20%

of the plants' photosynthate, translates to a huge amount of carbon worldwide. Thus, the AM symbiosis also plays a significant role in carbon cycling between the atmosphere and biosphere (Bago et al. 2000; Zhu and Miller 2003). The perfect example of obligate symbionts is an AM fungus which totally depends on plant roots for reducing carbon, and in return they provide numerous benefits such as uptake of nutrients, etc (Entry et al. 2002). AM fungi take about 20% of photosynthetic product which allocate by plants and roughly equal to 5 billion tonnes of carbon provide to AM fungi by plants (Dahlgren et al. 2004; Ganry et al. 1985). In contrast to this important phenomenon, some mycoheterotrophic plants consume their source from mycorrhizal fungi. The mycorrhizal fungi actively play role in nutrient cycling; they also help in absorbing the nutrients from the soil as nitrogen and phosphate uptake and reduce the biotic (pathogens of root) and abiotic (heavy metals, salinity, and drought) stress of the host plant (Mohammadi 2011). In endomycorrhizas, no sheath is formed, and the fungi colonize the root cortex both intercellularly and intracellularly.

Ectotrophic and arbuscular mycorrhizal interactions are highly beneficial economically, and their applications and ecological significances are also very high. AM fungi colonize the roots of many agriculturally important plants (food and bioenergy crops). They may serve as biofertilizers and bioprotectors against pathogens and toxic stresses in environmentally sustainable agriculture (Bücking et al. 2002). Ectomycorrhizal fungi on the other hand colonize a smaller number of plant species, but play as symbiotic partners of tree and shrub species as a key role in forest ecosystems (Finlay 2008), and could be a critical component in phytoremediation or revegetation applications (Bücking 2011; Giri et al. 2007). In many cases, individual plants may found to be infected by multiple strains of mycorrhizal fungi (Gherbi et al. 2008; Akiyama et al. 2010; Maillet et al. 2011; Bonfante and Requena 2011) which increase host-plant growth.

10.3 Ecological Aspects

In accordance with the evolutionary history, AM symbioses can be found in almost all ecosystems. They have been described from deserts (Corkidi and Rincón 1997; Dalpé et al. 2000; Titus et al. 2002), tropical rainforests (Brundrett et al. 1999; Guadarrama and Alvarez-Sanchez 1999; Siqueira and Saggin-Júnior 2001; Zhao et al. 2001; Gaur and Adholeya 2002), aquatic environments (Khan 1993), as well as from ecosystems with strong saline (Carvalho et al. 2001; Sengupta and Chaudhuri 2002a, b), sodic, or gypsum soils (Landwehr et al. 2002). The relatively low number of plants colonized by AM fungi in some arctic and antarctic habitats seems to be due to a lack of suitable vectors for fungal spores rather than to other causes (Allen 1996). In addition to the global distribution of AM symbioses, there is large functional diversity as well. Whereas most AM symbioses are mutualistic, a growing number of non-photosynthetic plants are described, which are receiving a large portion of their nutrients from AM fungi (Imhof 1999; Yamato 2001),

resembling the functioning of orchid mycorrhizas (Rasmussen 2002). In some cases, these mycotrophic plants are living epiparasitically on other plants using the hyphae of their fungal partner for the transfer of nutrients (Bidartondo et al. 2002). On the other hand, AM fungi may become parasitic themselves in relation to their host plant under special circumstances (Allen 1996).

Fossil record suggests that the mycorrhizal symbiosis originated from the Ordovician, 450–500 million years ago (Redeker et al. 2000), and they also performed an important role in filling land with plants. The journey of mycorrhizal fungi starts with the germination of spore and growth of the fungal hyphae toward a host root (Martin and Nehls 2009). The cell of the plant prepares its intracellular environment (Handelsman 2004). For the exchange of nutrients, the parenchyma cortex of plant is primarily attacked by fungus.

There are two groups of Mycorrhizal fungi known till this era, viz., aseptate endophytes such as Glomeromycota and septate such as Basidio and Ascomycota (Smith and Read 2008). Anatomically, mycorrhizae are broadly of three major types: ectomycorrhizas, ectendomycorrhizas, and endomycorrhizas, which depend upon the colonization of mycorrhizal fungi on the root intercellular spaces or develop inside the cell (Bonfante and Genre 2008).

10.4 AM Fungi

The AM fungi are obligate biotrophs and depend entirely on the plant to provide them with carbon. It's considered to be asexual, although the hyphae of genetically distinct strains can anastomose and exchange genetic material (Hijri and Sanders 2005; Croll et al. 2009). Our inability to grow AM fungi in the absence of the plant has impeded the study of these organisms, and in comparison with other groups of fungi, relatively little is known about them. When not in association with a plant, AM fungi exist in the soil as resting spores, which in some species are large enough to be visible with the naked eye (Schüßler et al. 2001). Currently, little is known about their genetics or the organization of their genomes. Their resting spores are multinucleate, and analyses of the ribosomal DNA sequences of many species indicated unusually high levels of polymorphism at these loci (Clapp et al. 2001; Kuhn et al. 2001). Initially, AM fungi were classified as zygomycetes, and the morphological characteristics of their spores were used as taxonomic markers (Morton and Benny 1990). Recently, analyses of the small subunit rRNA sequences led to a reclassification and the creation of a new phylum, the Glomeromycota, a sister clade to the Ascomycota and Basidiomycota (Schüßler et al. 2001; Kramadibrata et al. 2000). Some analyses suggested that they are heterokaryotic, whereas other studies predicted that they are homokaryotic (Kuhn et al. 2001; Pawlowska and Taylor 2004). Estimation of genome sizes for these fungi varies greatly and most indicated large genomes (Hosny et al. 1998). In contrast, a recent study found that *Glomus intraradices*, a species that has been maintained in coculture with excised roots for many years (Bécard and Fortin 1988), has a haploid

genome of 15 Mb (Hijiri and Sanders 2004). The genome of this species is now being sequenced, which will provide significant insights into this ancient, obligate symbiont.

10.5 Organizational Features of AM Plant Roots and Fungal Life Cycle

AM fungi are obligate biotrophs and rely on their autotrophic host to complete their life cycle and to produce spores which are able to germinate without the presence of a host, but the spores respond with an increase in hyphal branching and metabolic activity to root exudates (Bücking et al. 2008; Gachomo et al. 2009; Tamasloukht et al. 2003). Plant roots release strigolactones like substances that are able to induce pre-symbiotic growth of AM fungal spores (Akiyama et al. 2010).

On the host root surface, AM fungi form a specific appressorium (hyphopodium) from this hyphopodium; fungal Hyphae penetrate into the root through the pre-penetration apparatus, which guides the fungal hyphae from root cells up to the cortex. In the cortex, the hyphae enter the apoplast, and grow laterally along the root axis, and penetrate into inner root cortical cells (Fig. 10.2). In “typical” AM associations, the fungus enters the cell by small hyphal branches that continuously branched and develops into highly branched arbuscules. By contrast, in some cases, the mycorrhizas spread the fungus primarily from cell to cell and develops extensive intracellular hyphal coils that sometimes show an arbuscular like branching (Smith and Read 2008). The fungus does not enter the plant symplast and is excluded from the host cytoplasm by the enlarged periarbuscular membrane (PAM) of the host. Some fungi also form vesicles, fungal storage organs in the root apoplast (Bücking 2011).

Despite its coenocytic nature, the mycelium that is formed within the root, the intraradical mycelium (IRM) differs morphologically and functionally from the extraradical mycelium (ERM), the mycelium that grows into the soil (Bücking et al. 2012). The ERM absorbs nutrients from the soil and transfers these nutrients to the host root. The IRM on the other hand releases nutrients into the interfacial apoplast and exchanges them against carbon from the host. The fungus uses these carbon resources to maintain and enlarge the ERM, for cell metabolism such as active uptake processes, nitrogen assimilation, etc. and for the development of spores, which are able to initiate the colonization of a next generation of host plants (Bücking et al. 2012).

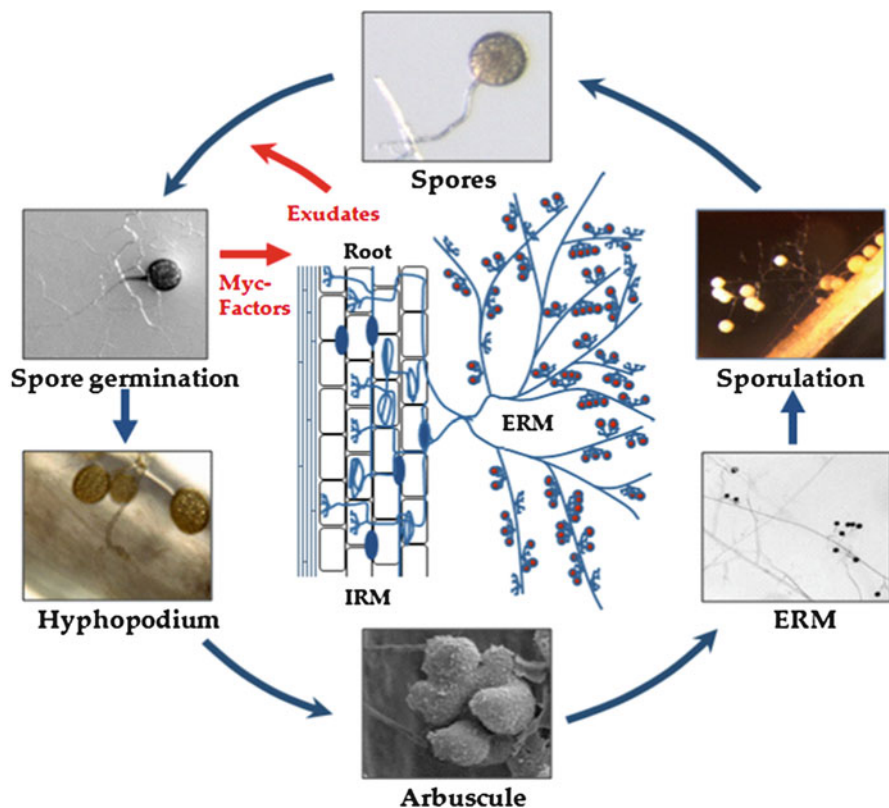


Fig. 10.2 Life cycle of an AM fungus and the different steps during AM development (Bücking et al. 2012)

10.6 Root Colonization with AM Fungi

Similar to Nod factors that play an important role in root nodulation, AM fungi release Myc factors that lead to an expression of plant symbiosis related genes and prepare the root for AM symbiosis. One active Myc factor has been identified as lipochitooligosaccharide (Maillet et al. 2011). Nod factors are also lipochitooligosaccharides and have a similar composition. It has been suggested that Nod factors developed from Myc factors, and that the functions of Myc and Nod factors overlap (Bonfante and Requena 2011). This is also supported by the fact that AM and rhizobial symbiosis share parts of the same signal transduction pathway—the so-called common symbiosis pathway. So far seven genes (SYM genes) of the common symbiosis pathway have been identified that are required for both root symbioses.

10.6.1 Plant Root Colonization and Morphological Changes

The process starts with germination (hyphal growth) of fungal spores, followed by poorly understood events. Subsequently, appressoria are formed from which the fungus penetrates the root surface and colonizes the intercellular space of the root cortex. On the fungal side, nonaggressive cell wall-lytic enzymes become active, and both the plant root cells and the fungus change their gene expression pattern and morphology. The hyphae penetrate the cell walls and develop within the cortex cells tree-like structures, called arbuscules, by repeated dichotomous branching. In some cases, intercellular storage organs, lipid-rich vesicles, and finally extraradical spores are formed, which may enter another colonization process. Fungal root colonization is under control of the plant aiming at a morphological and functional compatibility of the two partners (Bonfante and Perotto 1995). The key feature of AMs is the arbuscule, a highly branched haustorium-like structure within root cortex cells, responsible for nutrient exchange. However, the arbuscules represent a dead-end in the growth of AM fungi (Bonfante and Perotto 1995), and they finally senesce and collapse after 4–10 days of symbiosis (Sanders et al. 1977), possibly caused by the continuously stressful environment of the host cortex cell (Harley and Smith 1983).

Formation of arbuscules is accompanied by alterations in morphology of the host cell: the central vacuole is fragmented; the volume of cytoplasm and number of cell organelles increase significantly, and the nucleus moves into a central position and undergoes hypertrophy (Balestrini et al. 1994). The host cytoplasm and cell organelles proliferate around the branching hyphae. The number of plastids in colonized cortex cells increases (Bonfante and Perotto 1995) and networks are formed covering the arbuscules (Fester et al. 2001; Hans 2003). The plastids in these networks are connected to each other by so-called “stromules” (stroma-filled tubules) (Köhler and Hanson 2000). It has been shown that microtubules are involved in changes of host cell morphology and cytoplasmic architecture.

Four types of microtubule patterns were observed in arbusculated cells: (1) long bundles of microtubules crossing the cytoplasm among the arbuscule branches and passing through the arbuscule; (2) short microtubules connecting fine arbuscule branches or connecting arbuscule branches either to the cortical region of the cell or to the cell nucleus; (3) bundles of microtubules in the periphery (cortical region) of the host cell and along the hyphal trunk; and (4) perinuclear bundles of microtubules.

10.7 Mycorrhizal Interface in AM Interactions

The interface compartment that develops between the plant and the fungus is continuous with the peripheral plant cell wall (Bonfante and Perotto 1995). Although the fibrillar interface differs from the peripheral plant cell wall in

structure, its components reflect the composition of the wall of the host cell that is being invaded. This mixture of primary plant cell wall components indicates that the arbusculated plant cells have maintained their abilities to synthesize and secrete cell wall material. That this material does not assemble further to build up a secondary wall might be the result of lytic activities of the fungus (Peretto et al. 1995). When the arbuscule begins to senesce, the fibrillar material encapsulates the collapsed fungal structures that are then degraded completely by the plant cell. Subsequently, the cells regain their original morphology (Jacquelinet-Jeanmougin et al. 1987) and are able to allow another arbuscule formation.

Some processes of AM establishment are known to be mediated by phytohormones on the plant side, as suggested by application experiments (Barker and Tagu 2000). The levels of cytokinins are higher in shoots and roots of mycorrhizal plants compared to non-mycorrhizal ones (Allen et al. 1980). A possible role of abscisic acid was suggested from the fact that its level increases in AM roots (Danneberg et al. 1992; Bothe et al. 1994). Jasmonic acid applied exogenously promotes colonization and development of mycorrhizal structures (Regvar et al. 1996). The observed endogenous rise of jasmonates in barley roots correlating with mycorrhization, however, is more indicative for a role in AM (Hause et al. 2002).

Critical for the mutualism in the AM symbiosis is the bidirectional exchange of nutrients across the mycorrhizal interface. The interface between the fungus and the host includes the PAM and the fungal plasma membrane, the fungal cell wall, and the periarbuscular space between the fungal cell wall and the PAM. The PAM differs in its protein composition from the plant plasma membrane of non-arbusculated cells and is characterized by mycorrhiza-inducible transporters that facilitate the uptake of nutrients from the mycorrhizal interface. One of these transporters is Pt4, a high affinity phosphate (P) transporter that is only expressed in mycorrhizal roots and that is involved in the acquisition of P delivered by the fungus (Dewbre et al. 2002). A high-affinity ammonium (NH_4^+) transporter (AMT2;2) is also localized in the PAM. This transporter is exclusively expressed in arbusculated cells of mycorrhizal roots, but not in root nodules (Guether et al. 2009b). In contrast to other high affinity NH_4^+ transporters of plants, AMT2;2 of *Lotus japonicus* (LjAMT2;2) transfers NH_3 instead of NH_4^+ , and it has been suggested that the transporter takes up the positively charged NH_4^+ from the mycorrhizal interface and releases uncharged NH_3 into the plant cytoplasm. The detection of mycorrhiza-inducible sulfate transporters in AM roots suggests that also sulfate is transferred from the AM fungus to the host across the mycorrhizal interface (Casieri et al. 2012; Allen and Shachar-Hill 2009). The transport of carbon from the host to the fungus is driven by a monosaccharide transporter in the fungal arbuscular membrane (MST2) (Helber et al. 2011). This transporter takes up glucose but also other monosaccharides, such as xylose, what indicates that the fungus can also use cell wall sugars of the plant as alternative carbon source.

10.7.1 *The Ect-endomycorrhiza*

In ect-endomycorrhizas, the sheath may be reduced or absent; the Hartig net is usually well developed, but the hyphae penetrate into the cells of the plant. As already mentioned, the same species of fungus may form ectomycorrhizas on one species of plant and ect-endomycorrhizas on others. Arbutoid mycorrhizas possess sheath, external hyphae, and usually a well-developed Hartig net (Mohammadi 2011).

10.7.2 *The Ectomycorrhizal Fungi*

Ectomycorrhizas are very much important in numerous boreal and temperate forests which contributed approximately 30% of total microbial biomass of forest soils. Ectomycorrhizas are present in some families of woody gymnosperms (e.g., *Pinaceae*) and angiosperms (e.g., *Betulaceae*, *Dipterocarpaceae*). In ectomycorrhizas, the mycorrhizal fungi form a structure known as mantle (sheath) which encloses the rootlet (Mohammadi 2011).

Hyphae also penetrate inside the cells of the root to form a complex intercellular system, which appears as a network of hyphae in section known as the Hartig net, where a minute or no intracellular penetration takes place. In a few plants, the development of the Hartig net is negligible (Smith and Read 2008).

There are approximately 7000 to 10000 fungal species and 8000 plant species that form ectomycorrhizal (ECM) associations (Taylor and Peterson 2005). The number of plant species is relatively small (approximately 3%), but the group includes plants with high global and economic importance due to the disproportionate large terrestrial land surface that these plants cover and as main producers of timber. The plant species include wooden perennials, trees, or shrubs from cool, temperate boreal or montane forests, but also species from arctic alpine shrub communities (Smith and Read 2008; Tamasloukht et al. 2003). However, most of these plant species are not exclusively colonized by ECM fungi. Many species, such as *Populus*, *Salix*, *Betula* and *Fagus*, also form AM interactions, and there are indications that the AM symbiosis is the common mycorrhizal form of this taxon (Smith and Read 2008).

ECM fungi are relatively closely related to saprotrophic fungi and mainly belong to the Basidiomycota (e.g., *Amanita muscaria*, *Hebeloma cylindrosporum*, *Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius*, *Suillus bovinus*, *Xerocomus badius*), but also include some Ascomycota (e.g., *Cenococcum geophilum*, *Tuber borchii*, *Scleroderma hypogaeum*) (Smith and Read 2008). The switch from the presumably ancestral saprotrophic to the symbiotic behavior developed convergently in several fungal families during evolution. In contrast to AM fungi, many ECM fungi can be grown in axenic culture without a host, and this has allowed screening of their ability to use different carbon or nutrient sources

(Salzer et al. 1997). ECM fungi have a dual life style and are considered to be facultative saprotrophs. In the soil, they are highly competitive in nutrient acquisition and secrete a number of hydrolytic enzymes that allow them to degrade litter polymers and to use organic nutrient sources (Finlay 2008). At the same time they live within plant roots as symbionts, and this requires a set of adaptation mechanisms to avoid plant parasitism. ECM fungi have for example lost their ability to degrade plant cell wall polysaccharides (cellulose, pectins, and pectates), and this restricts their penetration into the root to the intercellular spaces (Martin and Martin 2010).

10.7.2.1 Root Colonization with Ectomycorrhizal Fungus

Typical for ECM roots are changes in the root morphology, such as the dichotomous branching of lateral roots, e.g., in pines, the production of a large number of root meristems and as a result an extensive root branching, inhibition of root hair formation, and the enlargement of cortical cells. Many of these morphological effects can be observed prior to colonization and can be interpreted as a preparation of the plant to increase root symbiosis.

Prior to the establishment of a functional ECM root and similar to the processes during AM development, there is an exchange of signals and cross-talk between both partners. The fungal tryptophan betaine hypaphorine has been shown to trigger reduced root hair elongation and swelling of the root hair tip and a stimulation of short root formation (Tamasloukht et al. 2003). ECM fungi also produce phytohormones, including auxins, cytokinins, abscisic acid, and ethylene, and it has been shown that the changes in the root morphology are caused by an overproduction of auxin in ECM fungal hyphae and changes in the endogenous hormone levels in the roots. The effect of ECM fungi on lateral root formation is independent from the plant's ability to form ECM associations. The ECM fungus *Laccaria bicolor* can induce lateral root formation also in *Arabidopsis thaliana*, a non-mycorrhizal plant, and the effect is correlated to an accumulation of auxin in the root apices (Felten et al. 2009). The auxin accumulation in the root tips and/or other fungal signals could stimulate basipetal auxin transport and lateral root primordia formation by an induction of plant genes involved in auxin transport and signaling.

The fungal partner responds to root exudate components, such as rutin and zeatin, with stimulation in hyphal growth and branching and growth towards the root and an accumulation of hypaphorine (Martin and Martin 2010). In response to host signals, ECM fungi also release effector proteins into the rhizosphere, such as nucleus after its uptake, and alter plant gene expression (Plett et al. 2011). *MiSSP7* has been shown to be crucial for the establishment of the ECM symbiosis and resembles effector proteins of pathogenic fungi and bacteria with similar function. A transcriptional response of the host can be observed within hours after an initial contact between both partners has been established. Plant genes encoding proteins involved in stress and defense response, as well as genes involved in signal

transduction and communication, and water uptake are upregulated in response to the presence of an ECM fungus in the rhizosphere (Sebastiania et al. 2009).

10.7.2.2 Organizational Characters of Ectomycorrhizal Roots

An established ECM symbiosis is characterized by three structural components: the hyphal sheath or mantle, the Hartig net (in later passages of this text sometimes also referred to as intraradical mycelium or IRM), and the extraradical mycelium. The hyphal sheath or mantle closes the root completely. The structural composition of the mantle is very diverse and can range from relatively thin, loosely arranged assemblages of hyphae to very thick, multilayered and pseudoparenchymatous mantles. The surface of the mantle can be compact and smooth or rough with numerous emerging hyphae and hyphal strands or rhizomorphs. The fungal sheath is involved in nutrient storage and controls the nutrient transfer to the host. The fungal mantle can represent a significant apoplastic barrier (Bücking et al. 2002; Ashford et al. 1988) and thereby creates a closed interfacial apoplast, in which the conditions can be controlled by both partners.

The Hartig net plays the key role in the nutrient transfer between both partners. The Hartig net is formed by hyphae that penetrate into the root cortex intercellularly. The penetration depth of the Hartig net differs between angiosperms and gymnosperms. Most angiosperms develop an epidermal Hartig net and confine the penetration of the Hartig net to the outer epidermis, which is often radially elongated. By contrast, the Hartig net in gymnosperms normally encloses several layers of cortical cells and sometimes extends up to the endodermis (Smith and Read 2008).

The extraradical mycelium (ERM) of the fungus acts as an extension of the root system, and it has been estimated that the ERM of the fungus *Pisolithus tinctorius* can represent 99% of the nutrient-absorbing surface length of pine roots (Ashford et al. 1988). The ERM of ECM fungi can account for 32% of the total microbial biomass and 700–900 kg ha⁻¹ in forest soils (Högberg and Högberg 2002). The ERM can have a relatively simple organization with individual hyphae with similar structure that grow into the soil (mainly in ascomycetes) or can be differentiated into singular hyphae and rhizomorphs. Rhizomorphs are aggregates of hyphae which grow in parallel and whose organization level can range from simple assemblages of undifferentiated and loosely woven hyphae to complex aggregations of hyphae with structural and functional differentiations (Agerer 2001).

10.7.2.3 Mycorrhizal Interface in Ectomycorrhizal Links

Transport studies suggest that in ECM associations, nutrients are exchanged simultaneously across the same interface (Bücking and Heyser 2001). The interface includes the plasma membranes and cell walls of both partners and the interfacial matrix between both partners. The plant transfers photosynthates as sucrose from

source to sink organs and ECM roots act as strong carbon sinks in mycorrhizal root systems. It is generally accepted that in contrast to phytopathogenic fungi or ericoid mycorrhizal fungi, AM and ECM fungi are not able to use sucrose as a carbon source, and that they take up simpler sugars, such as glucose or fructose, from the mycorrhizal interface. The presence of invertase genes in fungal genomes is correlated with the nutritional mode and in contrast to other plant-associated fungi, such as pathogens or endophytes, there are no indications that AM or ECM fungi possess invertase genes (Parrent et al. 2009) or have invertase activity (Salzer and Hager 1991). Consequently, mycorrhizal fungi rely on the invertase activity of the host in the interfacial apoplast for sucrose hydrolysis. Sucrose hydrolysis makes the hexoses glucose and fructose available for the fungus, and it has been suggested that glucose is mainly taken up by hyphae of the Hartig net and fructose mainly by hyphae of inner mantle layers (Nehls et al. 1998). Compared to the ERM, fungal hexose transporters are upregulated in ECM roots, indicating that the fungus in symbiosis takes up carbon primarily from the mycorrhizal interface (Lopez-Pedrosa et al. 2006).

The high affinity NH_4^+ importer *AmAMT2* of *Amanita muscaria* is upregulated in the ERM, but downregulated in Hartig net and the fungal sheath (Willmann et al. 2007). The high expression of this transporter in the ERM suggests a high capability of the ERM for NH_4^+ uptake. The low expression level in the Hartig net on the other hand indicates that NH_4^+ can serve as a potential nitrogen source that is delivered by the mycorrhizal fungus to the host. A low expression level of this NH_4^+ importer in the Hartig net would reduce the re-absorption of NH_4^+ by the fungus from the interfacial apoplast and increase the net transport of NH_4^+ to the host. The potential transport of NH_4^+ across the ECM interface is also supported by the presence and upregulation of plant high affinity NH_4^+ importers in ECM roots (Selle et al. 2005).

10.8 Phosphorus Uptake Improvement

The AM symbiosis is a highly compatible association, and in phosphate-limiting conditions, intraradical development of the fungus can occur in more than 80% of the root length. In addition to the intraradical growth phase, the fungus also maintains an extraradical mycelium that can extend several centimeters from the root (Mohammadi 2011). The fungal hyphae within the root are connected to the extraradical mycelium and form a single continuum. The extraradical hyphae acquire phosphate, initiate the colonization of other roots, and, in most species, are also the site of sporulation. Phosphate is delivered to the plant across the arbuscule cortical cell interface, and, recently, plant phosphate transporters involved in this process were identified (Harrison et al. 2002; Paszkowski et al. 2002; Rausch and Bucher 2002). Although not proven directly, it is anticipated that carbon is taken up by the arbuscule. The arbuscule cortical cell interface shares some structural and functional similarities with the endosymbiotic interfaces of other plant–microbe endosymbioses including the symbiosome, the symbiotic

interface of the rhizobium-legume symbiosis, and the haustorial-plant interface formed by the biotrophic fungal pathogens (Smith and Smith 1997; Parniske 2000).

Under conditions of low P availability, which occur in many soils, the AMF mediated transfer of nutrients has been reported from the host plant to another plant. Hyphae of mycorrhizas may spread from one infected plant and enter the roots of one or more other plants (Heap and Newman 1980). It has been shown that assimilates may be transported from one plant to another through AM hyphal connections. In a study, transfer of ^{14}C photosynthate from one plant to another was found primarily through AM hyphae rather than leakage from the roots of the donor plants. Similar results were obtained in a ^{32}P experiment, where hyphal linkage between plants was the dominant factor for transferring P (Chiariello et al. 1982). Ganry et al. (1985) conducted an experiment to investigate the effect of P fertilization on AM colonization and BNF, and based on the results of a preliminary pot study, field site is selected with a low colonization potential. In the absence of P fertilizer or in the presence of insoluble rock phosphate, there were no significant differences in AM colonization between -AM and +AM treatments, but when soluble superphosphate fertilizer was applied, AM colonization of inoculated roots at 26 days was greater than for the -AM treatment. These early differences disappeared by day 40 with AM frequencies 490% in all treatments. As mentioned, the most prominent effect of AMF is to improve P nutrition of the host plant in soils with low P levels due to the large surface area of their hyphae and their high affinity P uptake mechanisms. To substantiate this concept of plant growth promotion by AMF, several studies have shown that AM fungi contribute to up to 90% of plant P demand (Vander Heijden et al. 1998).

10.9 Mitigation of Environmental Stresses

Soils rarely provide ideal conditions for growth and survival of plants and soil microorganisms. Since soil conditions are constantly changing, the soil environment may favor development of arbuscular mycorrhizas at one point in time and inhibit them at another time. AM fungi have an important role in promotion of biological and chemical properties of plants under stressed environment (Mohammadi 2011). AM help plants to adapt to and resist a wide range of biotic and abiotic stresses they encounter in the environment. Adequate soil moisture and temperature may favor development of arbuscular mycorrhizas. However, when soil moisture or temperature becomes too high or low, mycorrhizal formation may be inhibited. AMF may alter the metal concentration in plants by metal immobilization in intra- or extra-radical hyphal cell wall components, metal chelation by fungal secreted compounds, such as glomalin, or by metal compartmentalization inside fungal cells. Thus, these AMF act as metal sinks, reducing local concentrations in soils and creating a more suitable environment for plants growing in soils with high metal contents. At molecular level, some reports show that the expression of plant genes related to metal tolerance was altered by mycorrhizal colonization

(Andrade et al. 2010). Arbuscular mycorrhizal fungi may affect host plant function and productivity under both high and low moisture conditions in greenhouse studies; drought-stressed maize infected with *Glomus mosseae* had higher concentrations of glucose, fructose, and total amino acids in leaves and roots than non-mycorrhizal plants (Schenck and Smith 1982). After applying periods of drought stress of varying length and severity, arbuscular mycorrhizal colonization increased leaf area, total plant and root biomass, number of tillers, and grain yield of wheat.

The arbuscular mycorrhizal symbiosis may alleviate plant responses to moderate moisture deficit by several mechanisms including increased water uptake from the soil by hyphae, altered hormonal levels, causing changes in stomatal conductance, increased turgor by lowering leaf osmotic potential, improved nutrition of the host, and improved plant recovery after drought by maintaining the soil-root continuum (Entry et al. 2002). AM fungi can enhance plant growth under salinity stress, especially in soils with low level of P and are able to enhance plant tolerance under salinity through altering plant physiology and increasing water and nutrient uptake. For example, mycorrhizal plants absorbed less amounts of Na^+ and Cl^- or inhibit their transfer to the shoots resulting in the increased dry weight of cotton by 68% under the salinity of 3 g/kg (Tian et al. 2004).

10.10 Mitigation of Heavy Metals Stress

Heavy metal uptake and tolerance depend on both plants and soil factors including soil microbes; therefore, information on interactions between plant roots and their symbionts such as AM fungi is required in order to understand heavy metal effects. Only few plants (the metallophytes) can cope with the adverse conditions on heavy metal soils. Availability and toxicity of metals to plants and mycorrhizal fungi varies, depending on the actual concentrations and oxidation states of the metals; soil and rhizosphere pH; and soil cation exchange capacity, CEC, texture, organic matter content, and redox potential. In roots, metals such as aluminum can impair cell division, increase cell wall rigidity, alter root respiration, precipitate nucleic acids, and interfere with the uptake and transport of Ca, Mg, P, and Fe. Fungal hyphae sequester metals, which may serve to reduce movement into and toxicity to the host stress tolerance. Detoxification mechanisms enable the plant and fungus to avoid toxic effects (Entry et al. 2002). Most reports note a positive effect of mycorrhizal inoculation on growth of plants in metal-contaminated soils. This protective benefit may be related to the adsorptive or binding capability for metals of the relatively large fungal biomass associated with host plant roots, which may physically minimize or exclude the entry of metals into host plants.

Several biological and physical mechanisms have been proposed to explain the generally lower metal toxicity to plants colonized by arbuscular mycorrhizal fungi. These include adsorption onto plant or fungal cell walls present on and in plant tissues or onto or into extraradical mycelium in soil (Joner et al. 2000; Meharg and

Cairney 2000) chelation by such compounds as siderophores and metallothioneins released by fungi or other rhizosphere microbes and sequestration by plant-derived compounds like phytochelatins or phytates. Other possible metal tolerance mechanisms include dilution by increased root or shoot growth, exclusion by precipitation onto polyphosphate granules, and compartmentalization into plastids or other membrane-rich organelles (Entry et al. 2002).

Metallophytes have developed various different physiological adaptations which enable them to compete successfully with the harsh conditions in heavy metal soils.

In addition, protection by AMF that colonize plant roots and considerably reduce the uptake of heavy metals into plant cells may be one of the means that allow metallophytes to thrive on heavy metal-polluted sites (Ouziad et al. 2005; Vogel-Mikus et al. 2006). For example, both zinc violets are strongly colonized by AMF, and leaves of *Viola lutea* ssp. *calaminaria* collected from a heavy metal site were earlier found to contain low amounts of heavy metals in ranges similar to those detected in non-metallophytes. This correlation is not likely to be coincidental, since mycorrhizal colonization of the roots increases with increasing heavy metal content of the soil. Since under adverse conditions, AM might be more important for plant metal resistance and under the optimized conditions of normal agricultural practice; however, AM colonization even could increase plant absorption from polluted soil and cleansed polluted sites by removing aboveground parts. It is suggested that metal-tolerant mycorrhizal inoculants might be considered for soil reclamation; thus, *G. caledonium* might be a promising mycorrhizal fungus for bioremediation of heavy metal-contaminated soil (Mohammadi 2011).

10.11 Conclusions

It should be apparent from the preceding discussion that different types of mycorrhizal symbioses show fundamental aspects in the frame of the terrestrial ecosystems and that the distinctive plant communities lead the major terrestrial biomes of the today's world because different kinds of symbiotic associations have been favored by selection that are adapted functionally to prevalently lodging of climatic and edaphic conditions characterizing different environments. The primary producer (plants) of an ecosystem is connected by mycorrhizal fungi which are its main significance to the distribution of required nutrients for their growth and also facilitate the flow of energy needed for nutrient mobilization and translocation of mobilized products backward to their hosts. This process enlightens the way of mycorrhizal fungi role in regulating the biogeochemical cycles. Old views of mycorrhizal symbiosis that are entirely based on the mineral nutrition of individual plants are hence giving way to new theories with extensive functional basis, making use of major ecologically relevant species and substrates. Comparative analysis of diverse systems will enhance our understanding of responses to environmental and climatic perturbations. New molecular tools have empowered identification of mycorrhizal fungal symbionts with more advance degree of resolution and have

contributed to the realization that the degree of functional specificity in mycorrhizal associations may be much greater than hitherto appreciated. This new knowledge is an imperative prerequisite for future, sustainable management of terrestrial ecosystems.

Acknowledgment Authors are grateful to DBT for partial financial assistance and DST for providing Confocal Microscope.

References

- Agerer R (2001) Exploration types of ectomycorrhizae a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107–114
- Akiyama K, Ogasawara S, Ito S, Hayashi H (2010) Structural requirements of strigolactones for hyphal branching in AM fungi. *Plant Cell Physiol* 51:1104–1117
- Allen MF (1996) The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peak into the 21st. *Mycol Res* 100:769–782
- Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. *Plant Physiol* 149:549–560
- Allen MF, Moore TS Jr, Christensen M (1980) Phytohormone changes in *Bouteloua gracilis* infected by vesicular–arbuscular mycorrhizae. *Can J Bot* 58:371–374
- Andrade SAL, Gratao PL, Azevedo RA, Silveira APD, Schiavinato MA (2010) Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. *Environ Exp Bot* 68:198–207
- Ashford AE, Peterson CA, Carpenter JL, Cairney JWG, Allaway WG (1988) Structure and permeability of the fungal sheath in the pisonia mycorrhiza. *Protoplasma* 147:149–161
- Bago B, Pfeffer PE, Shachar-Hill Y (2000) Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiol* 124:949–957
- Balestrini R, Romera C, Puigdomenech P, Bonfante P (1994) Location of a cell wall hydroxyproline-rich glycoprotein, cellulose and β -1,3-glucans in apical and differentiated regions of maize mycorrhizal roots. *Planta* 195:201–209
- Barker SJ, Tagu D (2000) The roles of auxins and cytokinins in mycorrhizal symbioses. *J Plant Growth Regul* 19:144–154
- Bearden BN, Petersen L (2000) Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of a vertisol. *Plant Soil* 218:173–183
- Bécard G, Fortin JA (1988) Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytol* 108:211–218
- Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Dominguez L, Sersic A, Leake JR, Read DJ (2002) Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* 26:345–346
- Bonfante P (2001) At the interface between mycorrhizal fungi and plants: the structural organization of cell wall, plasma membrane and cytoskeleton. In: Hock B (ed) *Mycota, IX fungal associations*. Springer, Berlin, pp 45–91
- Bonfante P, Anca I (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annu Rev Microbiol* 63:363–383
- Bonfante P, Genre A (2008) Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. *Trends Plant Sci* 13:492–498
- Bonfante P, Perotto S (1995) Strategies of arbuscular mycorrhizal fungi when infecting host plants. *New Phytol* 130:3–21

- Bonfante P, Requena N (2011) Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis. *Curr Opin Plant Biol* 14:451–457
- Bothe H, Klingner A, Kaldorf M, Schmitz O, Esch H, Hundeshagen B, Kernebeck H (1994) Biochemical approaches to the study of plant-fungal interactions in arbuscular mycorrhizas. *Experientia* 50:919–925
- Brundrett MC, Abbott LK, Jasper DA (1999) Glomalean mycorrhizal fungi from tropical Australia. I: comparison of the effectiveness and specificity of different isolation procedures. *Mycorrhiza* 8:305–314
- Bücking H (2011) Ectomycorremediation: an eco-friendly technique for the remediation of polluted sites. In: Rai M, Varma A (eds) Diversity and biotechnology of ectomycorrhizae. Soil biology, vol 25. Springer, Berlin, pp 209–229
- Bücking H, Heyser W (2001) Microautoradiographic localization of phosphate and carbohydrates in mycorrhizal roots of populus tremulax populus alba and the implications for transfer processes in ectomycorrhizal associations. *Tree Physiol* 21:101–107
- Bücking H, Kuhn AJ, Schröder WH, Heyser W (2002) The fungal sheath of ectomycorrhizal pine roots: an apoplastic barrier for the entry of calcium, magnesium, and potassium into the root cortex? *J Exp Bot* 53:1659–1669
- Bücking H, Abubaker J, Govindarajulu M, Tala M, Pfeffer PE, Nagahashi G, Lammers P, Shachar-Hill Y (2008) Root exudates stimulate the uptake and metabolism of organic carbon in germinating spores of glomus intraradices. *New Phytol* 180:684–695
- Bücking H, Liepold E, Ambilwade P (2012) The role of the mycorrhizal symbiosis in nutrient uptake of plants and the regulatory mechanisms underlying these transport processes. *Plant Sci*:107–138. doi:10.5772/52570
- Cappellazzo G, Lanfranco L, Fitz M, Wipf D, Bonfante P (2008) Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*. *Plant Physiol* 147:429–437
- Cardon ZG, Whitbeck JL (2007) The rhizosphere. Elsevier Academic Press, Burlington, p 235
- Carvalho SM, Cador I, Martins-Lou A (2001) Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza* 11:303–309
- Casieri L, Gallardo K, Wipf D (2012) Transcriptional response of *Medicago truncatula* sulphate transporters to arbuscular mycorrhizal symbiosis with and without sulphur stress. *Planta* 235:1431–1447
- Chiariello N, Hickman JC, Mooney MA (1982) Endomycorrhizal role for interspecific transfer of phosphorus in a community of annual plants. *Science* 217:941–943
- Clapp J, Rodriguez A, Dodd JC (2001) Inter- and intra-isolate rRNA large subunit variation in *Glomus coronatum* spores. *New Phytol* 149:539–554
- Corkidi L, Rincón E (1997) Arbuscular mycorrhizae in a tropical sand dune ecosystem on the Gulf of Mexico. I: mycorrhizal status and inoculum potential along a successional gradient. *Mycorrhiza* 7:9–15
- Croll D et al (2009) Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* 181:924–937
- Dahlgren RA, Saigusa M, Ugolini FC (2004) The nature properties and management of volcanic soils. *Adv. Agron.* 82:393–472
- Dalpé Y, Diop TA, Plenchette C, Gueye M (2000) Glomales species associated with surface and deep rhizosphere of *Faidherbia albida* in Senegal. *Mycorrhiza* 10:125–129
- Danneberg G, Latus C, Zimmer W, Hundeshagen B, Schneider-Poetsch H, Bothe H (1992) Influence of vesicular–arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L.). *J Plant Physiol* 141:33–39
- Deshmukh S, Huckelhoven R, Schäfer P, Imani J, Sharma M (2006) The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. *Proc Natl Acad Sci U S A* 103:50–57
- Dewbre GR, Harrison JA, Liu J (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14:2413–2429

- Entry JA, Rygielwicz PT, Watrud LS, Donnelly PK (2002) Influence of adverse soil conditions on the formation and function of Arbuscular mycorrhizas. *Adv Environ Res* 7:123–138
- Felten J, Kohler A, Morin E, Bhalerao RP, Palme K, Martin F (2009) The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar and *Arabidopsis* through auxin transport and signaling. *Plant Physiol* 151:1991–2005
- Fester T, Strack D, Hause B (2001) Reorganization of tobacco root plastids during arbuscule development. *Planta* 213:864–868
- Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extra radial mycelium. *J Exp Bot* 59:1115–1126
- Gachomo E, Allen JW, Pfeffer PE, Govindarajulu MD, Douds D, Jin HR, Nagahashi G, Lammers PJ, Shachar-Hill Y, Bücking H (2009) Germinating spores of glomus intraradices can use internal and exogenous nitrogen sources for de novo biosynthesis of amino acids. *New Phytol* 184:399–411
- Garay F, Diem HG, Wey J, Dommergues YR (1985) Inoculation with *Glomus mosseae* improves N₂ fixation by field-grown soybeans. *Biol Fertil Soils* 1:15–23
- Gaur A, Adholeya A (2002) Arbuscular–mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biol Fertil Soils* 35:214–218
- Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Gicze G, Auguy F, Peret B, Laplaze L, Franche C, Parniske M, Bogus D (2008) SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankia bacteria. *Proc Natl Acad Sci U S A* 105:4928–4932
- Giri B, Kapoor R, Mukerji KG (2007) Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum*, may be partly related to elevated K⁺/Na⁺ ratios in root and shoot tissues. *Microb Ecol* 54:753–760
- Guadarrama P, Alvarez-Sanchez FJ (1999) Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico. *Mycorrhiza* 8:267–270
- Guether M, Balestrini R, Hannah M, He J, Udvardi M, Bonfante P (2009a) Genome-wide reprogramming of regulatory networks, transport, cell wall and membrane biogenesis during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *New Phytol* 182:200–212
- Guether M, Neuhauser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P (2009b) A mycorrhizal specific ammonium transporter from *Lotus japonicus* acquires nitrogen. *Plant Physiol* 150:74–83
- Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev* 68:669–685
- Hans J (2003) Doctoral thesis, University Halle-Wittenberg, Halle, Germany. Academic Press, London
- Harley JL, Harley EL (1987) A check-list of mycorrhiza in the British flora. *New Phytol* 105:1–102
- Harley JL, Smith SE (1983) *Mycorrhizal symbiosis*. Academic Press, London
- Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. *Annu Rev Microbiol* 59:19–42
- Harrison MJ, Van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* 378:626–629
- Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14:2413–2429
- Hause B, Maier W, Miersch O, Kramell R, Strack D (2002) Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. *Plant Physiol* 130:1213–1220
- Heap AJ, Newman EL (1980) Links between roots by hyphae of vesiculararbuscular mycorrhizas. *New Phytol* 85:169–171

- Helber N, Wippel K, Sauer N, Saarschmidt S, Hause B, Requena N (2011) A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp. is crucial for the symbiotic relationship with plants. *Plant Cell* 23:3812–3823
- Hijiri M, Sanders IR (2004) The arbuscular mycorrhizal fungus *Glomus intraradices* is haploid and has a small genome size in the lower limit of eukaryotes. *Fungal Genet Biol* 41:253–261
- Hijiri M, Sanders IR (2005) Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature* 433:161–163
- Högberg MN, Högberg P (2002) Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytol* 154:791–795
- Holford ICR (1997) Soil phosphorus: its measurement and its uptake by plants. *Aust J Soil Res* 35:227–239
- Hosny M, Gianinazzi-Pearson V, Dullieu H (1998) Nuclear DNA contents of eleven fungal species in Glomales. *Genome* 41:422–428
- Imhof S (1999) Root morphology, anatomy and mycotrophy of the achlorophyllous *Voyria aphylla* (Jacq.) Pers. (Gentianaceae). *Mycorrhiza* 9:33–39
- Jacquelinet-Jeanmougin J, Gianinazzi-Pearson V, Gianinazzi S (1987) Endomycorrhizas in the gentianaceae. II: ultrastructural aspects of symbiont relationships in *Gentiana lutea* L. *Symbiosis* 3:269–286
- Javot H, Varma Penmetsa R, Terzaghi N, Cook DR, Harrison MJ (2007) A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci U S A* 104:1720–1725
- Joner EJ, Ravnskov S, Jakobsen I (2000) Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radio-labelled inorganic and organic phosphate. *Biotech Lett* 22:1705–1708
- Khan AG (1993) Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. *Mycorrhiza* 3:31–38
- Köhler RH, Hanson MR (2000) Plastid tubules of higher plants are tissue-specific and developmentally regulated. *J Cell Sci* 113:81–89
- Kramadibrata K, Walker C, Schwarzott D, Schussler A (2000) A new species of *Scutellospora* a with a coiled germination shield. *Ann Bot* 86:21–27
- Kuhn G, Hijiri M, Sanders IR (2001) Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* 414:745–748
- Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Tóth T, Biró B, Bothe H (2002) The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. *Mycorrhiza* 12:199–211
- Lopez-Pedrosa A, Gonzalez-Guerrero M, Valderas A, Azcon-Aguilar C, Ferrol N (2006) GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*. *Fungal Genet Biol* 43:102–110
- Lucic E, Fourrey C, Kohler A, Martin F, Chalot M (2008) Agene repertoire for nitrogen transporters in *Laccaria bicolor*. *New Phytol* 180:343–364
- Maillet F, Poinot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Cromer L, Giraudet D, Damien Formey D, Niebel A, Martinez EA, Driguez H, Bécard GJ (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* 469:58–63
- Martin NF, Martin F (2010) From galactic archaeology to soil metagenomic surfing on massive data streams. *New Phytol* 185:343–348
- Martin F, Nehls U (2009) Harnessing ectomycorrhizal genomics for ecological insights. *Curr Opin Plant Biol* 12:508–515
- Meharg AA, Cairney JWC (2000) Co-evolution of mycorrhizal symbionts and their hosts to metalcontaminated environments. *Adv Ecol Res* 30:69–112
- Mohammadi K (2011) Soil, plant and microbe interactions. Lambert Academic Publishing, Germany

- Morton JB, Benny GL (1990) Revised classification of arbuscular mycorrhizal fungi (zygomycetes): a new order, glomales, two new suborders, glomineae and gigasporineae, and two new families, acaulosporaceae and gigasporaceae, with an amendment of glomaceae. *Mycotaxon* 37:471–491
- Nehls U, Wiese J, Guttenberger M, Hampp R (1998) Carbon allocation in ectomycorrhizas: identification and expression analysis of an *Amanita muscaria* monosaccharide transporter. *Mol Plant Microb Interact* 11:167–176
- Newman EI, Reddell P (1987) The distribution of mycorrhizas among families of vascular plants. *New Phytol* 106:745–751
- Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends Ecol Evol* 10:407–411
- Ouziad F, Hildebrandt U, Schmelzer E, Bothe H (2005) Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. *J Plant Physiol* 162:634–649
- Parniske M (2000) Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease? *Curr Opin Plant Biol* 3:320–328
- Parent JL, James TY, Vasaitis R, Taylor AFS (2009) Friend or foe? Evolutionary history of glycoside hydrolase family 32 genes encoding for sacrolytic activity in fungi and its implications for plant–fungal symbioses. *BMC Evol Biol* 9:148–164
- Paszowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci U S A* 99:13324–13329
- Pawlowska TE, Taylor JW (2004) Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* 427:733–737
- Peretto R, Bettini V, Favaron F, Alghisi P, Bonfante P (1995) Polygalacturonase activity and location in arbuscular mycorrhizal roots of *Allium porrum* L. *Mycorrhiza* 5:157–163
- Pirozynski KA, Malloch DW (1975) The origin of land plants: a matter of mycotropism. *Biosystems* 6:153–164
- Plett JM, Kempainen M, Kale SD, Kohler A, Legue V, Brun A, Pardo TAG, Martin FA (2011) A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Curr Biol* 21:1197–1203
- Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) *Mycorrhiza*. Springer, Switzerland, pp 1–7
- Rasmussen HN (2002) Recent developments in the study of orchid mycorrhiza. *Plant Soil* 244:149–163
- Rausch C, Bucher M (2002) Molecular mechanisms of phosphate transport in plants. *Planta* 216:23–37
- Redeker D, Kodner R, Graham L (2000) Glomalean fungi from the ordovician. *Science* 289:1920–1921
- Regvar M, Gogala N, Zalar P (1996) Effects of jasmonic acid on mycorrhizal *Allium sativum*. *New Phytol* 134:703–707
- Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc Natl Acad Sci U S A* 91:11841–11843
- Requena N, Perez-Solis E, Azcon-Aguilar C, Jeffries P, Barea JM (2001) Management of indigenous plant–microbe symbioses aids restoration of desertified ecosystems. *Appl Environ Microbiol* 67:495–498
- Rillig MC, Maestre FT, Lamit LJ (2003) Microsite differences in fungal hyphal length, glomalin, and soil aggregate stability in semiarid Mediterranean steppes. *Soil Biol Biochem* 35:1257–1260
- Salzer P, Hager A (1991) Sucrose utilization of the ectomycorrhizal fungi *Amanita muscaria* and *Hebeloma crustuliniforme* depends on the cell wall-bound invertase activity of their host *Picea abies*. *Bot Acta* 104:439–445

- Salzer P, Hubner B, Sirrenberg A, Hager A (1997) Differential effect of purified spruce chitinases and β -1,3-glucanases on the activity of elicitors from ectomycorrhizal fungi. *Plant Physiol* 114:957–968
- Sanders FE, Tinker PB, Black RLB, Palmerley SM (1977) The development of endomycorrhizal root systems I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. *New Phytol* 78:257–268
- Schachtman DP, Reid RJ, Ayling SM (1998) Phosphorus uptake by plants: from soil to cell. *Plant Physiol* 116:447–453
- Schenck NC, Smith GS (1982) Responses of six species of vesicular-arbuscular mycorrhizal fungi and their effects on soybean at four soil temperatures. *New Phytol* 92:193–201
- Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the glomeromycota: phylogeny and evolution. *Mycol Res* 105:1413–1421
- Schüßler A, Martin H, Cohen D, Fitz M, Wipf D (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. *Nature* 444:933–936
- Sebastiana M, Figueiredo A, Acioli B, Sousa L, Pessoa F, Balde A, Pais MS (2009) Identification of plant genes involved on the initial contact between ectomycorrhizal symbionts (*Castanea sativa*-European chestnut and *pisolithus tinctorius*). *Eur J Soil Biol* 45:275–282
- Selle A, Willmann M, Grunze N, Gessler A, Weiss M, Nehls U (2005) The high-affinity poplar ammonium importer *PttAMT1.2* and its role in ectomycorrhizal symbiosis. *New Phytol* 168:697–706
- Selosse MA, Roy M (2009) Green plants that feed on fungi: facts and questions about mixotrophy. *Trends Plant Sci* 14:64–70
- Sengupta A, Chaudhuri S (2002a) Arbuscular mycorrhizal relations of mangrove plant community at the Ganges river estuary in India. *Mycorrhiza* 12:169–174
- Sengupta A, Chaudhuri S (2002b) Arbuscular mycorrhizal relations of mangrove plant community at the Ganges river estuary in India. *Mycorrhiza* (4):169–174
- Simon L, Bousquet J, RC L, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363:67–69
- Siqueira JO, Saggin-Júnior OJ (2001) Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. *Mycorrhiza* 11:245–255
- Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*. Academic Press, San Diego, CA
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*. Academic Press, New York
- Smith FA, Smith SE (1997) Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *New Phytol* 137:373–388
- Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol* 133:16–20
- Strack D, Fester T (2006) Isoprenoid metabolism and plastid reorganization in arbuscular mycorrhizal roots. *New Phytol* 172:22–34
- Tamasloukht MB, Sejalou-Delmas N, Kluever A, Jauneau A, Roux C (2003) Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea*. *Plant Physiol* 131:1468–1478
- Taylor JH, Peterson CA (2005) Ectomycorrhizal impacts on nutrient uptake pathways in woody roots. *New Forests* 30:203–214
- Thompson JN, Cunningham BM (2002) Geographic structure and dynamics of co-evolutionary selection. *Nature* 417:735–738
- Tian CY, Feng G, Li XL, Zhang FF (2004) Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Appl Soil Ecol* 26:143–148
- Titus JH, Titus PJ, Nowak RS, Smith SD (2002) Arbuscular mycorrhizae of Mojave desert plants. *West N Am Nat* 62:327–334

- Vander Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72
- Vogel-Mikus K, Pongrac P, Kump P, Necemer M, Regvar M (2006) Colonisation of a Zn, Cd and Pb hyper accumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environ Pollut* 139:362–371
- Willmann A, Weiss M, Nehls U (2007) Ectomycorrhiza, ectomycorrhiza-mediated repression of the high-affinity ammonium importer gene *AmAMT2* in *amanita muscaria*. *Curr Genet* 51:71–78
- Yamato M (2001) Identification of a mycorrhizal fungus in the roots of achlorophyllous *Sciaphila tosaensis* Makino (Triuridaceae). *Mycorrhiza* 11:83–88
- Zhao ZW, Xia YM, Qin XZ, Li XW, Cheng LZ, Sha T, Wang GH (2001) Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. *Mycorrhiza* 11:159–162
- Zhu YG, Miller RM (2003) Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends Plant Sci* 8:407–409

Chapter 11

The Management of the Mycorrhizal Soil Infectivity: Ecological and Technical Approaches

Adrien Lies, Yves Prin, Robin Duponnois, and Hicham Ferhout

Abstract Arbuscular Mycorrhizal Fungi have a large potential to help increase global food security. They constitute the most important microbial symbiosis for the majority of terrestrial plant species. Their ecological functions in the productivity and stability of agroecosystems have been recognized for many years. Many studies have shown that these symbionts improved plant growth and plant resistance to biotic and abiotic stresses. Despite the proven potential of mycorrhizal symbiosis to sustainably improve the productivity of agroecosystems, this biotechnology is still under exploited. This failure mainly results from technical difficulties to mass-produced fungal inoculum of high quality and a lack of knowledge about the biological factors regulating the soil receptivity of arbuscular mycorrhizal inoculation. In order to promote mycorrhizal soil infectivity, two main approaches could be considered: (1) the “reductionist” approach that consists to add into the soil, a large quantity of fungal propagules of a specialized AMF and (2) the “holistic” approach that aims to conserve and restore native AMF diversity and abundance. In this chapter, we will examine the environmental factors that affect the mycorrhizal diversity and abundance and limit both approaches as they can both be of interest, trying to explain to what environmental solution they would be more adapted.

A. Lies

AGRONUTRITION, Parc Activestre, 3 avenue de l’Orchidée, 31390 Carbonne, France

IRD, UMR LSTM, 34398 Montpellier, France

Y. Prin

CIRAD, UMR LSTM, 34398 Montpellier, France

R. Duponnois (✉)

IRD, UMR LSTM, 34398 Montpellier, France

e-mail: Robin.Duponnois@ird.fr

H. Ferhout

AGRONUTRITION, Parc Activestre, 3 avenue de l’Orchidée, 31390 Carbonne, France

11.1 Introduction

Producing enough food to feed a global human exceeding 7 billion, and estimates to reach 9 billion by 2050, has become the main challenge leading to a global crop yield increases of up to 100% (Godfray et al. 2010). The current global productivity of agrosystems is largely not efficient enough to reach these objectives of such yield increases. In addition, increasing crop resource use efficiency (yield per unit of resource input) has to be achieved by following the recommendations of the “doubly green revolution” that combines the objectives of the Green Revolution and the maintenance of biological diversity and ecosystem resilience but without decreases of actual yields. Hence, it becomes urgent to identify new technologies and better apply long-known agricultural practices (Bennett et al. 2013).

The main environmental factors that limit increasing crop yields are the poor soil fertility and particularly the availability of nitrogen and phosphorus (Tilman et al. 2002). Among the soil microbial components, it is well known that the arbuscular mycorrhizal fungi represent a potential low-input solution to increasing the overall yield of important staple crops resulting from their positive impacts on phosphate acquisition, water stress, or disease resistance (Rodriguez and Sanders 2015).

About 80% of all plant species, including most agricultural crops, can form arbuscular mycorrhizal (AM) symbiosis in all major terrestrial ecosystems (Opik et al. 2006). AM symbiosis (Fig. 11.1) plays a major role in soil fertility and plant nutrition and in the maintenance of stability and biodiversity within plant communities (Smith and Read 2008).

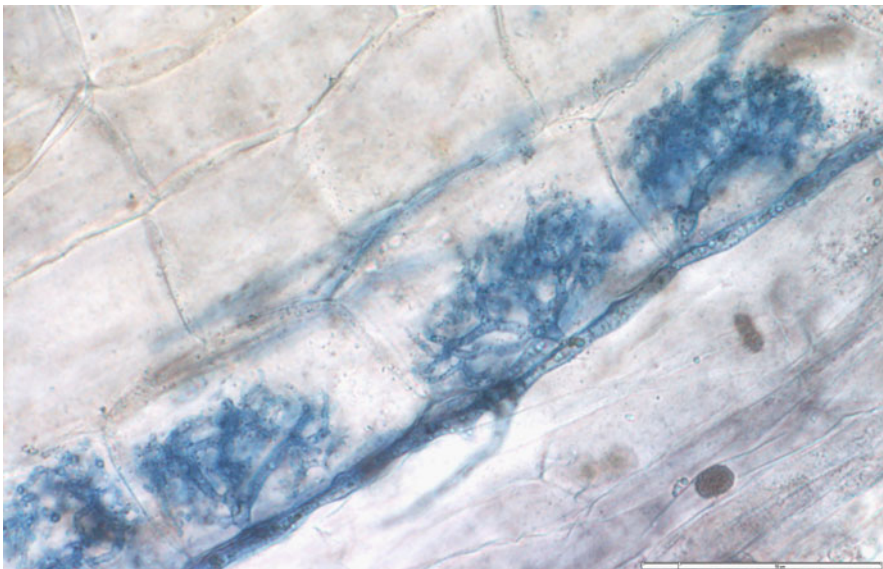


Fig. 11.1 Typical intracellularly formed arbuscules of an AMF within the cortical cells of *Tagetes* roots. Arbuscules are the main site of exchange between the plant and the fungus

AM fungi facilitate plant nutrient uptake and transport of less mobile soil nutrients (i.e., phosphorus) (Jakobsen et al. 2001), promote drought tolerance (Kaya et al. 2003), and limit pathogenic infections (Del Fabbro and Prati 2014). Although AM fungi (AMF) have been traditionally considered as non-host specific in their ability to infect plants, benefits resulting from AM symbiosis establishment for each partner could be highly dependent on the particular species involved (Burrows and Pflieger 2002). It has been reported that AMF taxa can differ significantly in their growth strategies (Hart and Reader 2002) and in their impact on plant growth and development (Klironomos 2003). In particular, AMF species or AMF assemblages mediate plant interspecific competition and plant community structure and diversity (van der Heijden et al. 1998; Klironomos et al. 2011; Koorem et al. 2012). In parallel, it has been shown that host species and species mixture impacted individual fungal species or fungal assemblages (Eom et al. 2000; Johnson et al. 2004).

Obviously, in the current context of development of an environment friendly agriculture, it has been suggested that the integration of key natural processes (i.e., facilitation, plant soil feedback) in agricultural practices could be an efficient strategy for agricultural management. These natural processes with significant potential for plant stress resistance and plant mineral nutrition are mainly subjected to the arbuscular mycorrhizal establishment (Fester and Sawers 2011). Hence, the management of these symbioses is of prime interest in practices of controlled fallows or crop successions, mixed crop cultures (e.g., cereals/legume associations), or agroforestry.

Although the potential of AMF to contribute to improved crop yields has been demonstrated for decades and despite the current knowledge on AMF establishment and loss, there are a lot of limitations that reduce the efficiency of this biotechnological approach. For instance, one of the main obstacles to AMF large-scale uses is their non-cultivability and availability as a pure microbial inoculant, as easy to use and apply as chemical fertilizers. In fact, if a number of commercial inoculants can be found worldwide, they represent, at the best, a very low taxonomic diversity limited to a few strains or species with more or less presumed wide plant spectrum compatibility. However, the recent emergence of massive soil microbiota sequencing has evidenced an extremely high taxonomic diversity among Glomeromycetes, with a lot of undescribed clusters, and a range of putative associated functions to be exploited in a smart agriculture.

Two main approaches could be considered: (1) the “*reductionist*” approach that consists to add into the soil, a large quantity of fungal propagules of a specialized AMF like in commercial AMF inoculants and (2) the “*holistic*” approach that aims to conserve and restore native AMF diversity and abundance (Fester and Sawers 2011). In the holistic approach, it is proposed to take benefit of the ability of some plant species like legumes or some aromatic plant species to associate and multiply a wide range of AMF partners. Introducing such plants in agricultural practices may considerably diversify the mycorrhizal soil potential and benefits to the associated crops.

Although nearly all soils are inhabited by indigenous AMF (Abbott and Robson 1982), their distribution and their abundance (i.e., the indigenous inoculum potential) show large variations within regions, soil types, and crop production systems (Gianinazzi-Pearson et al. 1985). In this chapter, we will consider the environmental factors that affect the mycorrhizal establishment and limit both approaches as they can both be of interest, trying to explain to what environmental solution they would be more adapted.

11.2 Environmental Factors that Affect AM Establishment and Efficiency

All cultivated soils contain diverse communities of AMF and, globally, all the important food crops are naturally colonized by AMF independently from mycorrhizal inoculation. Since the expected impact of AMF introduction is to increase plant productivity, a qualitative and quantitative assessment of the AMF community composition has to be performed before the application of fungal inoculum. Hence and in order to optimize the expected impact of the mycorrhizal symbiosis, an important starting point is to determine at what level AMF are limiting to the crop yield. These limitations have to be considered at least at two different parameters: abundance and diversity. It has been reported that abundance of AMF can be negatively affected by tillage, high levels of nutrients, and frequent fallow periods in the intensive agricultural production resulting in an insufficient root colonization and, consequently, a lower mycorrhizal effect on plant growth (Karasawa and Takebe 2011).

The other form of limitation concerns the meaning levels of AMF diversity. The main differences between natural ecosystems and agroecosystems are recorded in nutrient cycling and biological diversity. It has been reported that AMF diversity was higher in natural systems than in agricultural systems (Verbruggen et al. 2010) and that agricultural soils harbored a few select taxa within the AMF order Glomerales (Oehl et al. 2010). The poor biological diversity of current agroecosystems and its consequences on crop yield could be alleviated by using fungal inocula with low sensitivity for such agricultural practices.

11.3 The “Reductionist” or Microbe-targeted Approach: AM Fungi Isolation, Purification, Multiplication, and Application

In the soil, AMF are found as hyphae and spores. Spores can be separated from a soil sample by sieving and gradient centrifugation (Fig. 11.2) and separated in sublots according to their size (generally ranging from 10 to 1200 μm) and color.

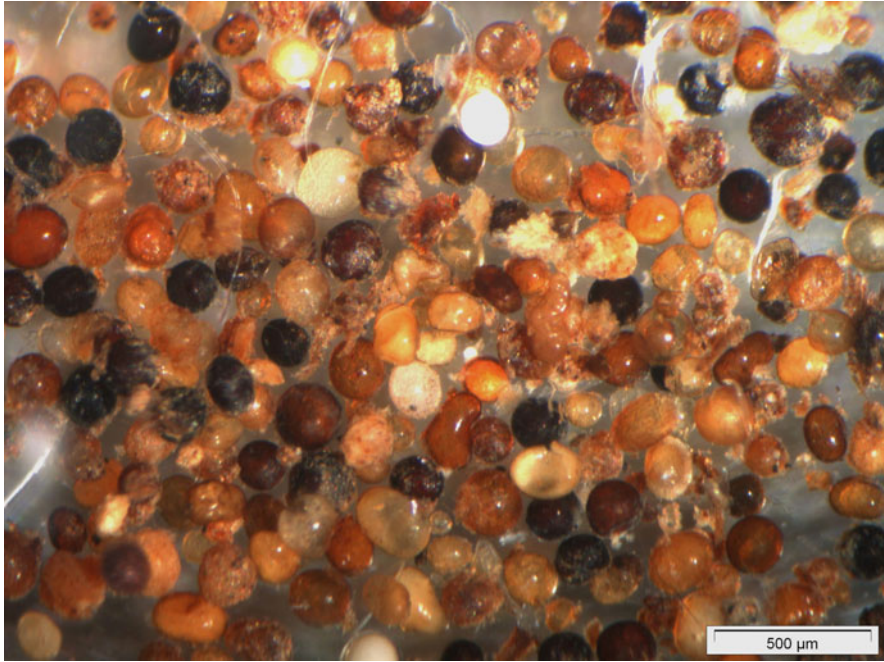


Fig. 11.2 A crude mix of AMF spores after wet sieving and sucrose gradient separation from a soil sampled in Morocco. Note the diversity of shape, size, and color

After this first separation, spores have to be manually and individually ranked under a stereomicroscope. A first taxonomic assignment may then be done from spore characteristics: color size, morphology cell wall organization, etc.

In a second step, spores can be multiplied on an easy-to-cultivate mycotrophic plant species (Tagetes, sorghum, leek, onion, maize, Bahia grass, etc.) in controlled conditions, and new mycorrhizal roots can then be used to multiply the fungi by re-inoculation of these sheared root systems on new plants. This inoculum allows to evaluate the mycorrhizal potential of the new AMF strain and can be molecularly identified by PCR/sequencing. Various cultural substrates could be used for propagation and large-scale production of AMF such as disinfected sandy soil, peat, vermiculite, perlite, and calcinated clay (Ijdo et al. 2011)

Alternatively, this multiplication step can be made on in vitro cultured *Agrobacterium*-transformed roots of carrot or alfalfa, according to Declerck et al. (1996), following surface-sterilization of spores. With this technical practice, it is possible to obtain fungal DNA free of DNA of other organisms (Koch et al. 2004).

Unfortunately, this last step may not be equally feasible with any AMF taxa, and fungal subculturing may be intrinsically blocked at any moment for an undetermined reason. Slow growth is generally observed with AMF and may constitute an open door to a wide range of fungal or bacterial contaminants stopping the multiplication step. Aside these difficulties, some strains like MUCL46238

Fig. 11.3 Monoxenic sporulation of the AMF strain MUCL46238 of *Rhizophagus clarus*, associated to transformed roots of carrot



(*Rhizophagus clarus*) (Fig. 11.3) are quite easy to multiply in microbiologically controlled conditions. They constitute the basis of most biologically active commercial AMF inoculants. Another AMF, *Rhizophagus irregularis*, is easily cultured in axenic conditions (Bécard and Fortin 1988), and its genome has recently been sequenced (Tisserand et al. 2013). The importance of this mycorrhizal fungus for the future applications of this fungal symbiont to improve food security is recognized principally because of: (1) its worldwide distribution and (2) its ability to be easily and efficiently cultured in axenic conditions (Rodriguez and Sanders 2015). Many field studies have reported its beneficial effect on yields of a globally important crop (Table 11.1).

To be compatible with field uses on large-scale applications, AMF strains' spores or sheared roots have to be embedded in or mixed with different types of substrate (peat, perlite, . . .) or polymer (alginate, gums, . . .) (Fig. 11.4) allowing their manipulation, survival, and field application to soil and plants. Such inocula are being used with success in different countries with various crops.

A number of studies and patents exist that compare the type and quality of different inoculant technologies, including seed coating or embedding (Malusá et al. 2012).

Diversifying the microbial offer of inoculants, i.e., mixing several AMF strains and possibly associating bacteria may be an efficient strategy to enhance the positive impact on plant growth. Some studies have reported that close interactions

Table 11.1 Effect of *R. irregularis* inoculation on the growth of different crop species in field conditions

Plant species	Biomass yield	Fruit yield	References
Wheat cv. Tetra	+22.1%	+22.4%	Babana and Antoun (2006)
Wheat	+23%	+7.7%	Wahbi et al. (2015)
Wheat	+15.4%	+13.4%	Suri et al. (2011)
<i>Trifolium alexandrinum</i>	+51.7%	–	Pellegrino et al. (2011)
Maize (Pioneer “3025W”)	+68.1%	+22.4%	Franco et al. (2013)
Maize	+53.6%	–	Celebi et al. (2010)
Maize	–	+44.6%	Hagh et al. (2016)
<i>Solanum lycopersicum</i> cv. Ercole	+19.6%	+29%	Conversa et al. (2013)
Maize (Zheng Dan 958)	+5%	+12.6%	Li et al. (2013)

⁽¹⁾nd: not determined

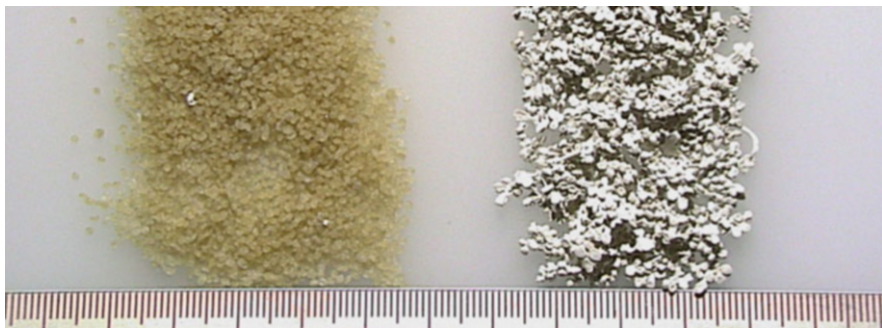


Fig. 11.4 Two formulations of an alginate-embedded inoculant with either pure (*left*) or 5% kaolin-supplemented (*right*) alginate. Kaolin is intended to stabilize the relative humidity level and improve survival rate of the microbial strain

occurred between AMF and rhizobacteria underlying the existence of a trophic complex where multitrophic interactions take place between AMF, mycorrhizosphere microbiota, and host plants (Duponnois et al. 2011). The beneficial traits of root-colonizing bacteria and mycorrhizal fungi have been frequently studied separately. However, it is now well known that synergistic effects of bacteria and mycorrhizal fungi occurred with respect to their combined beneficial impacts on plant growth (Vessey 2003). For instance, recent studies have shown that inoculation of PGPR with AMF is more beneficial in promoting plant growth compared to inoculation with either one of them (Ratti et al. 2001; Gamalero et al. 2004). In addition, it has been reported that rhizobacteria can improve the mycorrhizal establishment by stimulating the growth of fungal hyphae through the production of compost like vitamin or enzymes increasing the permeability of the cell wall of the root epidermis (Jeffries et al. 2003), but also indirectly at the “molecular dialogue” preceding the mycorrhizal symbiosis. These bacteria have been named Mycorrhization Helper Bacteria, MHB (Duponnois and Garbaye 1991). Some companies are commercializing such composite inoculants; however,

the technical elaboration of mixed inoculants associating AMF and other plant growth promoting rhizobacteria may be a brake in terms of quality, constancy, and cost.

11.4 The Holistic Approach: Taking Advantage of the Symbiotic Dependency of Some Plant Species

At this stage, an efficient alternative could be the selection and use of plants with particularly high level of mycotrophy, *i.e.*, that will recruit a diversified range of AM fungi and their associated bacteria directly in soils. Combining the direct use of plants and their associated microbial communities (also called “nurse plants” (Duponnois et al. 2013) or “holobionts” (Vandenkoornhuysen et al. 2015) as planted fallows or directly in association with the targeted crops may be an efficient way of introducing a diversified microbial inoculant. Among candidate mycotrophic plants are species of the *Crotalariae*, within the nitrogen-fixing legume (Fabaceae) family. Among these tropical plants, some species (*Crotalaria juncea* (Fig. 11.5), *C. grantiana*, *C. spectabilis*,...) produce alkaloids with a nematocidal activity, altogether these properties making them to be highly appreciated in the tropics as fallows producing green manure and nematode control.

Regarding AMF, Germani and Plenchette (2004) demonstrated that all *Crotalaria* species they tested significantly responded to AM inoculation with *Glomus intraradices* (now *Rhizophagus irregularis*), resulting in increased plant P content and plant growth. Moreover, as reported for other legumes (Medina-Gonzales et al. 1987), *Crotalaria* species showed their high mycorrhizal dependency, up to 90%, and their potential to increase the level of beneficial mycorrhizal fungi in soil (Germani and Plenchette 2004).

Other candidate plants include aromatic like *Lavandula* or *Thymus* that can be co-planted in Morocco with trees like *Cupressus* to improve tree growth and survival after field plantation (Duponnois et al. 2011).

Considering the symbiotic characteristics of plant species and varieties in the pluriannual and rehabilitation design of crops is also a way of maintaining the biological quality of soils. For example, plants of the brassicaceae (rapeseed, mustard), chenopodiaceae (spinach), or amaranthaceae (beet) are non-mycorrhizal (*i.e.*, non symbiotic); cereals like wheat or corn are mycorrhizal; and legumes like bean, faba, or alfalfa are both mycorrhizal and nitrogen-fixing species. Such characteristics were more or less empirically considered in traditional agriculture and are regaining interest in agro-ecological practices.

Additionally, a number of studies have shown that enhancing aboveground (plant) diversity has a positive impact on belowground (microbial) diversities and this is true for AMF. In this context, multispecies cropping systems may often be considered as a practical application of key ecological processes depending on biodiversity, plant interactions, and diverse natural regulation mechanisms that

Fig. 11.5 *Crotalaria juncea* used as a fallow in a vegetable production farm during a greenhouse trial in Noves (France)



ensure the productivity, resistance to disruption, and ecological sustainability of the agro-systems, especially in Mediterranean areas (Vandermeer 1989). For instance, the association of trees with crops (agro-forestry) allowed nutrient recycling by coexisting plant species exploring different soil depths increasing nutrient and water-use efficiency by the crops (van Noordwijk et al. 1996) (Fig. 11.6) Moreover and in these cropping systems, tree species could be considered as beneficial microbe inoculum sources for inter-row crop species (e.g., mycorrhizal fungi, PGPR, etc.) (Haselwandter and Bowen 1996).



Fig. 11.6 The association of *Argania spinosa* and peas in a vegetable farm near Agadir (Morocco). This agro-forestry system associating perennial oleaginous trees and short-term crops optimize land use, soil depth exploration, soil microbial activity, and diversify sources of cash incomes

11.5 Conclusion

This chapter has reviewed the large potential of AMF to help increase global security resulting from their positive impact on the development of all globally important food crops. However, despite numerous studies focused on this symbiosis, few data are available on large-scale inoculation experiments performed on important crops. According to the scientific knowledge that shows the complexity of the interactions between the plant, the physico-chemical characteristics of the soil and the soil microbiota, the traditional focus on nutrient exchange and plant growth response in order to evaluate the “symbiotic efficiency” in field conditions seems too simplistic to expect a large-scale application of AMF ensuring significant increases in food production. To enhance chances of successful inoculation, research efforts should have to be directed towards the impact of the introduction of nonnative AMF on the composition of resident AMF communities (i.e., distribution of inocula in large geographical areas, risk of “outbreeding depression” resulting from genetic exchange between the introduced exotic AMF strain and the native AMF strains, etc). Better knowledge is also required on the indirect effects on plant growth resulting from AMF inoculation that are not related to mycorrhizal root colonization. Hence and in order to make commercial application of AMF, a sum of scientific results have to be acquired especially to predict under which environmental conditions AMF inoculation (the reductionist approach) will

promote yield and agricultural sustainability compared to the “holistic approach” that will manage the mycorrhizal soil infectivity through an adequate cultural practice (i.e., agroforestry, intercropping, rotation).

References

- Abbott LK, Robson AD (1982) The role of vesicular–arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. *Aust J Agric Res* 33:389–408
- Babana AH, Antoun H (2006) Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (*Triticum aestivum* L.) in Mali. *Plant Soil* 287:51–58
- Bécard G, Fortin JA (1988) Early events of vesicular–arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytol* 108:211–218
- Bennett AE, Daniell TJ, White PJ (2013) Benefits of breeding crops for yield response to soil microorganisms. In: de Bruijn FJ (ed) *Molecular ecology of the rhizosphere*. Wiley, New York, NY, pp 17–27
- Burrows RL, Pflieger FL (2002) Arbuscular mycorrhizal fungi respond to increasing plant diversity. *Can J Bot* 80:120–130
- Celebi SZ, Demir S, Celebi R, Durak ED, Yilmaz IH (2010) The effect of Arbuscular Mycorrhizal Fungi (AMF) applications on the silage maize (*Zea mays* L.) yield in different irrigation regimes. *Eur J Soil Biol* 46:302–305
- Conversa G, Lazzizzera C, Bonasia A, Elia A (2013) Yield and phosphorus uptake of a processing tomato crop grown at different phosphorus levels in a calcareous soil as affected by mycorrhizal inoculation under field conditions. *Biol Fertil Soils* 49:691–703
- Declerck S, Strullu DG, Plenchette C (1996) In vitro mass-production of the arbuscular mycorrhizal fungus, *Glomus versiforme*, associated with Ri T-DNA transformed carrot roots. *Mycol Res* 100:1237–1242
- Duponnois R, Garbaye J (1991) Effect of dual inoculation of Douglas fir with the ectomycorrhizal fungus *Laccaria laccata* and mycorrhization helper bacteria (MHB) in two bare root forest nurseries. *Plant Soil* 138:169–176
- Duponnois R, Ouahmane L, Kane A, Thioulouse J, Hafidi M, Boumezzough A, Prin Y, Baudoin E, Galiana A, Dreyfus B (2011) Nurse shrubs increased the early growth of *Cupressus* seedlings by enhancing. *Soil Biol Biochem* 43:2160–2168
- Duponnois R, Ramanankierana H, Hafidi M, Baohanta R, Baudoin E, Thioulouse J, Sanguin H, Bâ AM, Galiana A, Bally R, Lebrun M, Prin Y (2013) Native plant resources to optimize the performances of forest rehabilitation in Mediterranean and tropical environment : some examples of nursing plant species that improve the soil mycorrhizal potential. *C R Biol* 336:265–272
- Del Fabbro C, Prati D (2014) Early responses of wild plant seedlings to arbuscular mycorrhizal fungi and pathogens. *Basic Appl Ecol* 15:534–542
- Eom AH, Hartnett DC, Wilson GWT (2000) Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia* 122:435–444
- Fester T, Sawers R (2011) Progress and challenges in agricultural applications of arbuscular mycorrhizal fungi. *Crit Rev Plant Sci* 30:459–470
- Franco AD, Ortiz FEC, Contreras MGL, Santacruz GAA, Cabrera OAG (2013) Growth, mineral absorption and yield of maize inoculated with microbe strains. *Afr J Agric Res* 8:3764–3769
- Gamalero E, Trotta A, Massa N, Copetta A, Martinotti MG, Berta G (2004) Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. *Mycorrhiza* 14:185–192

- Germani G, Plenchette C (2004) Potential of *Crotalaria* species as green manure crops for the management of pathogenic nematodes and beneficial mycorrhizal fungi. *Plant Soil* 266:333–342
- Gianinazzi-Pearson V, Gianinazzi S, Trouvelot A (1985) Evaluation of the infectivity and the effectiveness of indigenous vesicular-arbuscular fungal populations in some agricultural soils in Burgundy. *Can J Bot* 63:1521–1524
- Godfray H CJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF et al (2010) Food security: the challenge of feeding 9 billion people. *Science* 327:812–818
- Hagh ED, Mirshekari B, Ardakani MR, Farahvash F, Rejali F (2016) Optimizing phosphorus use in sustainable maize cropping via mycorrhizal inoculation. *J Plant Nutr* 39:1348–1356
- Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytol* 153:335–344
- Haselwandter K, Bowen GD (1996) Mycorrhizal relations in trees for agroforestry and land rehabilitation. *For Ecol Manag* 81:1–17
- Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant bio-diversity, ecosystem variability and productivity. *Nature* 396:69–72
- Ijdo M, Cranenbrouck S, Declerck S (2011) Methods for large-scale production of AM fungi: past, present and future. *Mycorrhiza* 21:1–16
- Jakobsen I, Gazey C, Abbott LK (2001) Phosphate transfer by communities of arbuscular mycorrhizal fungi in intact soil cores. *New Phytol* 149:95–103
- Jeffries P, Gianinazzi S, Perotto S, Turanu K, Barea M (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil Soils* 37:1–16
- Johnson D, Vandenkoornhuysen PJ, Leake JR, Gilbert L, Booth RE, Grime JP, Young PW, Read DJ (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytol* 161:503–515
- Karasawa T, Takebe M (2011) Temporal or spatial arrangements of cover crops to promote arbuscular mycorrhizal colonization and P uptake of upland crops grown after nonmycorrhizal crops. *Plant Soil* 353:355–366
- Kaya C, Higgs D, Kirnak H, Tas I (2003) Mycorrhizal colonization improves fruit yield and water use efficiency in water (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. *Plant Soil* 253:287–292
- Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84:2292–2301
- Klironomos JN, Zobel M, Tibbett M, Stock WD, Rillig MC, Parrent JL, Moora M, Koch AM, Facelli JM, Facelli E, Dickie IA, Bever JD (2011) Forces the structure plant communities: quantifying the importance of the mycorrhizal symbiosis. *New Phytol* 189:366–370
- Koch AM, Kuhn G, Fontanillas P, Fumagalli L, Goudet J, Sanders JR (2004) High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *Proc Natl Acad Sci U S A* 101:2369–2374
- Koorem K, Saks U, Söber V, Uibopuu A, Opik M, Zobel M, Moora M (2012) Effects of arbuscular mycorrhiza on community composition and seedling recruitment in temperate forest understorey. *Basic Appl Ecol* 13:663–672
- Li H, Wang C, Li X, Xiang D (2013) Inoculating maize fields with earthworms (Aporectodea trapezoids) and an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) improves mycorrhizal community structure and increases plant nutrient uptake. *Biol Fertil Soils* 49:1167–1178
- Malusá E, Sas-Paszt L, Ciesielska J (2012) Technologies for beneficial microorganisms inocula used as biofertilizers. *Sci World J*:1–12
- Medina-Gonzales OA, Sylvia DM, Jr K (1987) Growth response of tropical forage legumes to inoculation with *Glomus intraradices*. *Trop Grasslands* 21:24–27

- Van Noordwijk M, Lawson G, Soumaré A, Groot JJR, Hairiah K (1996) Root distribution of trees and crops: competition and/or complementary. In: Ong CK, Huxley PW (eds) Tree-crop interactions: a physiological approach. CAB International, Wallington, UK, pp 319–364
- Oehl F, Laczko E, Bogenrieder A, Stahr K, Bosch R, Van der Heijden MGA, Sieverding E (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biol Biochem* 42:724–738
- Opik M, Moora M, Liira J, Zobel M (2006) Composition of root colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *J Ecol* 94:778–790
- Pellegrino E, Bedini S, Avio L, Bonari E, Giovanetti M (2011) Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean soil. *Soil Biol Biochem* 43:367–376
- Ratti N, Kumar S, Verma HN, Gautam SP (2001) Improvement in bioavailability of tricalcium phosphate to *Cymbopogon matinii* var. motia by rhizobacteria, AMF and *Azospirillum* inoculation. *Microbiol Res* 156:145–149
- Rodriguez A, Sanders IR (2015) The role of community and population ecology in applying mycorrhizal fungi for improved food security. *ISME J* 9:1053–1061
- Smith SE, Read DJ (2008) The mycorrhizal symbiosis. Academic Press, San Diego, CA
- Suri VK, Choudhary K, Chander G, Verma TS (2011) Influence of vesicular arbuscular mycorrhizal fungi and applied phosphorus on root colonization in wheat and plant nutrient dynamics in a phosphorus-deficient acid alfisol of Western Himalayas. *Commun Soil Sci Plant Anal* 42 (10):1177–1186
- Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. *Nature* 418:671–677
- Tisserand E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R et al (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proc Natl Acad Sci U S A* 110:20117–20122
- Vandenkoornhuise P, Quaiser A, Duhamel M, Le Van A, Dufresne A (2015) The importance of the microbiome of the plant holobiont. *New Phytol* 206:1196–1206
- Vandermeer JH (1989) The ecology of intercropping. Cambridge University Press, Cambridge
- Verbruggen E, Röhling WFM, Gamper HA, Kowalchuk GA, Verhoef HA, Van der Heijden MGA (2010) Positive effects of organing farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytol* 186:968–979
- Vessey KJ (2003) Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255:571–586
- Wahbi S, Prin Y, Maghraoui T, Sanguin H, Thioulouse J, Oufdou K, Hafidi M, Duponnois R (2015) Field application of the mycorrhizal fungus rhizophagus irregularis increases the yield of wheat crop and affects soil microbial functionalities. *Am J Plant Sci* 6:3205–3215

Chapter 12

Reactive Oxygen Species (ROS) Metabolism and Signaling in Plant-Mycorrhizal Association Under Biotic and Abiotic Stress Conditions

Manoj Nath, Deepesh Bhatt, Ram Prasad, and Narendra Tuteja

Abstract A stringent regulation between reactive oxygen species (ROS) generation and scavenging is an essential process that helps a plant to adaptively utilize ROS as a primary defense molecule against biotic and abiotic stress condition. ROS at lower level primarily acts as a signaling molecule regulating plant cellular processes that include plant-microbe interaction. However, ROS generated at higher levels often leads to the inhibition of cellular processes, thus consequently leading a detrimental effect in plant growth and homeostasis. Rhizosphere being the “chemical space” around the roots which proves to be biologically active zone for plant-microbe interactions forms a link responsible for mutual signaling in each of the partners. Moreover plant fitness is said to be enhanced by these symbiotic mycorrhizal associations which are known to alleviate detrimental effects caused by environmental stresses thereby enhancing overall plant growth and development. This present chapter summarizes a precise interlink between biotic-abiotic stressed plants and its mycorrhizal association linking ROS modulation with plant signaling thereby establishing a link between stress tolerance and ROS metabolism. The literature reviewed herein will help to delineate the basic mechanism of ROS signaling, by ascertaining the physiological responses via altering the ROS metabolism, in mycorrhizal-associated stressed plants. This will ultimately help in designing innovative strategies to improve the overall plant productivity under stressful regimes.

M. Nath • R. Prasad • N. Tuteja (✉)

Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Sector 125, Noida 201313, India

e-mail: ntuteja1@amity.edu

D. Bhatt

Department of Biotechnology, Shree Ramkrishna Institute of Computer Education and Applied Sciences, Affiliated to Veer Narmad South Gujarat University, Surat, Gujarat 395001, India

12.1 Introduction

Reactive oxygen species (ROS) are generated in response to stress which includes both abiotic and biotic stress conditions as well as in normal metabolic processes, e.g., in chloroplast and mitochondrial electron transport chains. Enzymatic components are known to serve as one of the major ROS scavenging systems in plants, e.g., monodehydroascorbate reductase (MDHAR), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which are predominantly distinguished as one of the major players of the detoxification pathway. On the other hand, glutathione (GSH) and ascorbic acid (AsA) are often demarcated as components of nonenzymatic system (Gill and Tuteja 2010; Rasool et al. 2013). Additionally, respiratory burst oxidase homologues (RBOHs) and NADPH oxidases are also known to be major components of ROS production system in plants (Suzuki et al. 2013; Kadota et al. 2015).

However, in response to stress, ROS generation acts as a signaling agent that aware the plant for stress adaptations (Mittler et al. 2011; Sewelam et al. 2016). Contrarily an accurate balance, between ROS scavenging and ROS generation system, is indispensable for the utilization of ROS as signaling molecule under stress (Baxter et al. 2014). However, long duration of stress results in an increased ROS level that further leads to oxidative stress, thus inhibiting the crucial cellular activities and cell viability (Gill and Tuteja 2010; Barna et al. 2012). Therefore, antioxidant signaling, redox homeostasis, and continuous generation/scavenging of ROS are designated as key components of stress signals (Bose et al. 2014; Jajic et al. 2015). ROS generation is also known to occur during early mycorrhiza-plant symbiotic interactions (Fester and Hause 2005; Tanaka et al. 2006; Puppo et al. 2013; Espinosa et al. 2014; Kiirika et al. 2014).

Plants often interact with several microbes in rhizosphere; however, among these interactions, some beneficial interactions are known to enhance plant growth and fitness. However, in present scenario, a relatively small number of beneficial plant-microbe interactions are well characterized and utilized (Farrar et al. 2014). Microbes are capable to alleviate the effect of environmental stress on plants via decreasing the stress impacts thus ultimately increasing the plant fitness (Schouteden et al. 2015; Doty 2016). On the other hand, a huge number of microbes interact with the plant root, in the rhizosphere, thus affecting plant growth and fitness (Mine et al. 2014). The major active region identified for plant root-microbe interactions is the edge between soil and roots, i.e., through mutual signaling that occurs during plant-microbial association (Evangelisti et al. 2014).

Several fungal species are reported which are capable in colonizing plant roots. Additionally research on plant-microbe interaction has majorly spotlighted the areas of plant-arbuscular mycorrhizal fungi (AMF) symbiosis and legume root-rhizobium interaction for nitrogen fixation and pathogenesis (Smith and Smith 2011; Oldroyd et al. 2011; Kachroo and Robin 2013; Farrar et al. 2014). Mycorrhizal fungi are also well recognized to facilitate nutrient transfer from soil, e.g.,

transfer of phosphorus and nitrogen in plants (Behie and Bidochka 2014). Furthermore, the symbiotic beneficial fungal counterpart is also known to be capable of increasing the plant fitness via modifying the chemical plasticity such as modulation in response to stress (Goh et al. 2013).

Regardless of the facts, research work related to link ROS metabolism and plant-mycorrhizal association under stress is still scanty. Therefore, an attempt has been made here to briefly summarize ROS metabolism including generation/scavenging, signaling, and homeostasis in connection with plant-microbe interactions. Interestingly, research on AMF-like fungi, *Piriformospora indica*, has recently depicted their role in plant growth promotion under stressful conditions which has consequently led to an increase in plant yield (Sherameti et al. 2008; Vadassery et al. 2009a, b; Varma et al. 2012; Cruz et al. 2013; Jogawat et al. 2013; Bakshi et al. 2014; Trivedi et al. 2016; Gill et al. 2016). Therefore, ROS metabolism linked with *P. indica*-plant root association under stress will also be briefly summarized.

12.2 ROS Metabolism and Plant-Mycorrhizal Association Under Stress Conditions

Plants continuously interact with microbes, e.g., mutualists and pathogens, that affect plant growth (Mine et al. 2014). Beneficial mutualists such as AMF are well reported in providing plant growth sustainability under different stress conditions (Muthukumar and Udaiyan 2010; Porcel et al. 2012; Tahat and Sijam 2012). ROS generated in both radical and non-radical forms includes superoxide radicals ($O_2^{\bullet-}$), perhydroxyl radical (HO_2^{\bullet}), and alkoxy radicals (RO^{\bullet}) which constitute radical form, while hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2) constitute non-radical forms. However, radicals prove to be highly toxic in nature when compared to non-radicals (Gill and Tuteja 2010; Sewelam et al. 2016). The role of mycorrhiza in ROS scavenging is established from the ROS metabolism study of AM-colonized roots of *Zea mays*, *Medicago truncatula*, and *Nicotiana tabacum* (Fester and Hause 2005). Likewise, AMF-colonized plants accumulated less ROS (H_2O_2) and malondialdehyde (MDA) than non-colonized olive plants which ultimately helped in alleviating oxidative stress, thus increasing drought tolerance (Fouad et al. 2014). Similarly, AMF were also reported to enhance the antioxidant system of host plant and decrease the impact of drought stress condition in date palm (Benhiba et al. 2015) and *Citrus reticulata* (Sarkar et al. 2016).

AMF mediate the control of ROS metabolism and antioxidants and further diminish the effect of oxidative stress in host plants in response to stress conditions (Vos et al. 2013; Wu et al. 2014; Hashem et al. 2016). In addition, ROS involvement also suggests in providing tolerance against nematode (*Meloidogyne javanica*) infection in soybean (Beneventi et al. 2013). Similarly, root-knot nematode reduction was linked with ROS metabolism (*Meloidogyne incognita*) infection in mycorrhizal tomato roots (Vos et al. 2013). Increased antioxidant enzymes

including SOD, POD, CAT, APX, and GR were also argued to enhance cadmium (Cd) tolerance in tomato through AMF-mediated ROS scavenging activities (Hashem et al. 2016).

12.3 ROS Signaling

In order to adapt with various biotic and abiotic stresses, plants possess a highly complex signaling pathway. In addition, plants also utilize ROS as the major signaling agent and activate various adaptive defense mechanisms under stress conditions (Baxter et al. 2014; Xu and Brosche 2014; Sewelam et al. 2016). Higher expression of ROS scavenging-related genes, e.g., glutaredoxin, thioredoxin, and GPX, was correlated with herbicide (atrazine) stress tolerance in *Glomus mosseae/Medicago sativa*. Besides this, higher atrazine degradation was observed in *G. mosseae* (mycorrhizal)-treated *M. sativa* plants as compared with non-treated plants (Song et al. 2016).

However, ROS production is the most common response triggered which initiates signaling pathway under stress environment (Sewelam et al. 2016). A limited research related to ROS modulation during initial microbial interaction with plant root is available. ROS generation and cell death are also reported at the interaction site during early host-microbe association (Puppo et al. 2013). An active ROS component H₂O₂ is involved in adaptive defense mechanism which is also responsible for initiating signaling pathways in response to stressed environment (Xia et al. 2009). Due to its membrane-permeable nature, H₂O₂ further control the specific biological reactions providing stress tolerance in several components (Neill et al. 2002; Yan et al. 2007). The functional role of ROS through the role of exogenous H₂O₂ in regulating rhizobial symbiosis-related genes was demonstrated in *Medicago truncatula-Sinorhizobium meliloti* interactions (Andrio et al. 2013). On the other hand, a temporary ROS increase was also observed in root hairs of *Phaseolus vulgaris*, and specific ROS signature involvement was proposed during symbiotic association (Cardenas and Quinto 2008).

12.4 ROS Metabolism and Plant-*P. indica* Interaction Under Stress

A group of soil-dwelling fungi constitute AMF which are symbiotically associated with roots of many plants. Moreover, *P. indica*, AMF-like fungi, is an obligate biotroph which is able to be grown in pure culture and does not need the presence of the plant (Foley et al. 2011). It is well reported that *P. indica* has been found to improve plant growth and survival in agricultural, horticultural, and medicinal crops under stress condition (Verma et al. 1998; Waller et al. 2005; Baltruschat

et al. 2008; Vadassery et al. 2009a, b; Prasad et al. 2013; Jogawat et al. 2013; Lahrmann et al. 2013; Ye et al. 2014; Johnson et al. 2014; Gill et al. 2016; Trivedi et al. 2016). Interestingly, a potential role of *P. indica* has been also documented in plants in response to various kinds of biotic and abiotic stress including salt, drought, nutrient, and nematode stress tolerance (Sherameti et al. 2008; Cruz et al. 2013; Bakshi et al. 2014; Nath et al. 2016). In addition, *P. indica* was also reported to increase the alkaline phosphatase and acid phosphatase and consequently contributes for higher uptake of phosphate in plants (Malla et al. 2004).

In plant roots, ROS generation as a defense-related response was reported during initial plant-mycorrhizal associations (Pozo and Azcón-Aguilar 2007). Moreover, biotic stress tolerance was also linked with the ROS metabolism and modulation of antioxidant defense pathway in *P. indica*-inoculated plants, viz., wheat, maize, and barley (Waller et al. 2005; Serfling et al. 2007; Kumar et al. 2009). Besides this, ROS was also observed before *P. indica* physical contact with plant roots, though H_2O_2 was not reported after symbiotic relationship establishment (Vadassery et al. 2009a; Camehl et al. 2011; Vahabi et al. 2015). H_2O_2 was also found to encourage *OXII* (*oxidative signal-inducible1*) gene and further trigger defense response under pathogen infection (Rentel et al. 2004; Anthony et al. 2006; Petersen et al. 2009). Additionally, OXI1 (a serine/threonine kinase) was also demonstrated as a requirement for ROS-mediated responses and oxidative burst for disease tolerance against pathogens in *Arabidopsis* (Rentel et al. 2004; Petersen et al. 2009). H_2O_2 generation was also found to repress in *P. indica*-colonized *Arabidopsis* roots and growth stimulation mediated through PLD-PDK1-OXI1 pathway under favorable cocultivation conditions (Camehl et al. 2011). Exudates are released during initial interaction of *P. indica* with plant, which further leads to ROS accumulation and stomatal closure and induces defense-responsive genes in *Arabidopsis*. On the other hand, after the establishment of *P. indica*-plant interaction, the stomata are reopened, while ROS generation decreased and defense-responsive gene expression turned down (Vahabi et al. 2015).

Enhanced antioxidant system and glutathione-ascorbate cycle activation were observed in *P. indica*-colonized barley root (Waller et al. 2005). Similarly, *P. indica*-mediated enhancement of antioxidants was also linked with salinity stress tolerance in barley (Baltruschat et al. 2008). In another similar report in wheat, co-inoculation of *Azotobacter chroococcum* and *P. indica* indicated higher antioxidant enzyme activities including peroxidase and APX in colonized plants in response to zinc-deficient condition (Abadi and Sepehri 2016). Recently, candidate effector (PIIN_08944) expression of *P. indica* was reported to decline the ROS burst in barley (Akum et al. 2015). Here, we are summarizing the ROS metabolism link with plant-mycorrhiza interaction under stress (Fig. 12.1).

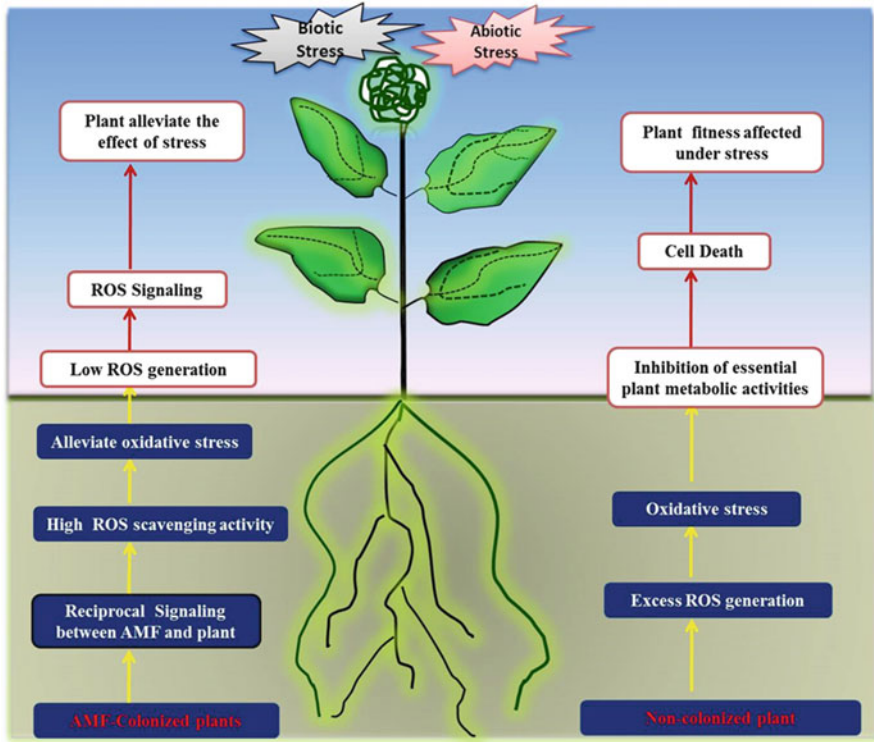


Fig. 12.1 An overview of ROS metabolism and plant-mycorrhizal association under stress. In response to biotic and abiotic stress, ROS metabolism and modulation offer adaptive defense stress response in mycorrhizal-colonized plants and further provide stress tolerance. On the other hand, in absence of colonization, high ROS generated and inhibit the essential cellular activities; therefore, the plant fitness is compromised. AMF, arbuscular mycorrhizal fungi

12.5 Conclusions

The capability of ROS metabolism defense system enhancement via symbiotic microbial interactions ultimately improves plant fitness under stress. A detailed ROS signature kinetics during initial plant-mycorrhiza interaction will enhance the basic understanding of mycorrhizal link with ROS metabolism. On the other hand, molecular insights of ROS metabolism in plant-mycorrhizal especially *P. indica* interaction will be very helpful to plan innovative approaches and ultimately to improve plant growth and yield under stress conditions.

References

- Abadi VAJM, Sepehri M (2016) Effect of *Piriformospora indica* and *Azotobacter chroococcum* on mitigation of zinc deficiency stress in wheat (*Triticum aestivum* L.) Symbiosis 69:9. doi:[10.1007/s13199-015-0361-z](https://doi.org/10.1007/s13199-015-0361-z)
- Akum FN, Steinbrenner J, Biedenkopf D, Imani J, Kogel K (2015) The *Piriformospora indica* effector PIIN_08944 promotes the mutualistic Sebacinalean symbiosis. Front Plant Sci 6:906. doi:[10.3389/fpls.2015.00906](https://doi.org/10.3389/fpls.2015.00906)
- Andrio E, Marino D, Marmeys A, de Segonzac MD, Damiani I (2013) Hydrogen peroxide-regulated genes in the *M. truncatula*–*Sinorhizobium meliloti* symbiosis. New Phytol 198:190–202. doi:[10.1111/nph.12120](https://doi.org/10.1111/nph.12120)
- Anthony RG, Khan S, Costa J, Pais MS, Bogre L (2006) The *Arabidopsis* protein kinase PTI-2 is activated by convergent phosphatidic acid and oxidative stress signaling pathways downstream of PDK1 and OXII. J Biol Chem 281:37536–37546
- Bakshi M, Sherameti I, Johri AK, Varma A, Oelmuller R (2014) Phosphate availability affects root architecture and development, plant performance and is controlled by root-colonizing microbes. J Endocyt Cell Res 25:56–65
- Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G et al (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol 180:501–510
- Barna B, Fodor J, Harrach BD, Pogany M, Kiraly Z (2012) The Janus face of reactive oxygen species in resistance and susceptibility of plants to necrotrophic and biotrophic pathogens. Plant Physiol Biochem 59:37–43
- Baxter A, Mittler R, Suzuki N (2014) ROS as key players in plant stress signalling. J Exp Bot 65:1229–1240. doi:[10.1093/jxb/ert375](https://doi.org/10.1093/jxb/ert375)
- Behie SW, Bidochka MJ (2014) Nutrient transfer in plant–fungal symbioses. Trends Plant Sci 19:1360–1385
- Beneventi MA, da Silva OB, de Sa MEL, Firmino AAP, de Amorim RMS, Albuquerque EVS et al (2013) Transcription profile of soybean–root-knot nematode interaction reveals a key role of phytohormones in the resistance reaction. BMC Genomics 10:322. doi:[10.1186/1471-2164-14-322](https://doi.org/10.1186/1471-2164-14-322)
- Benhiba L, Fouad MO, Essahibi A, Ghoulam C, Qaddoury A (2015) Arbuscular mycorrhizal symbiosis enhanced growth and antioxidant metabolism in date palm subjected to long-term drought. Trees 29:1725–1733
- Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. J Exp Bot 65:1241–1257
- Camehl I, Drzewiecki C, Vadassery J, Shahollari B, Sherameti I, Forzani C et al (2011) The OXII kinase pathway mediates *Piriformospora indica*-induced growth promotion in *Arabidopsis*. PLoS Pathog 7(5):e1002051. doi:[10.1371/journal.ppat.1002051](https://doi.org/10.1371/journal.ppat.1002051)
- Cardenas L, Quinto C (2008) Reactive oxygen species (ROS) as early signals in root hair cells responding to rhizobial nodulation factors. Plant Signal Behav 3:1–3
- Cruz C, Fegghi Z, Martins-Loucao MA, Varma A (2013) Plant nitrogen use efficiency may be improved through symbiosis with *Piriformospora indica*, Chap 17. In: Varma A et al (eds) *Piriformospora indica*. Soil Biology 33. doi:[10.1007/978-3-642-33802-1_17](https://doi.org/10.1007/978-3-642-33802-1_17), Springer, Berlin, pp 285–293
- Doty SL (2016) Plant–microbe symbiotic interactions. Plant Mol Biol 90:535. doi:[10.1007/s11103-016-0470-y](https://doi.org/10.1007/s11103-016-0470-y)
- Espinosa F, Garrido I, Ortega A, Casimiro I, Alvarez-Tinaut MC (2014) Redox activities and ros, no and phenylpropanoids production by axenically cultured intact olive seedling roots after interaction with a mycorrhizal or a pathogenic fungus. PLoS One 9(6):e100132. doi:[10.1371/journal.pone.0100132](https://doi.org/10.1371/journal.pone.0100132)

- Evangelisti E, Rey T, Schornack S (2014) Cross-interference of plant development and plant–microbe interactions. *Curr Opin Plant Biol* 20:118–126
- Farrar K, Bryant D, Cope-Selby N (2014) Understanding and engineering beneficial plant–microbe interactions: plant growth promotion in energy crops. *Plant Biotechnol J* 12:1193–1206
- Fester T, Hause G (2005) Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. *Mycorrhiza* 15:373–379
- Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, Mueller ND, O’Connell C, Ray DK, West PC, Balzer C, Bennett EM, Carpenter SR, Hill JL, Monfreda C, Polasky S, Rockstrom J, Sheehan J, Siebert S, Tilman D, David P, Zaks M (2011) Solutions for a cultivated planet. *Nature* 478:337–342
- Fouad OM, Essahibi A, Benhiba L, Qaddoury A (2014) Effectiveness of arbuscular mycorrhizal fungi in the protection of olive plants against oxidative stress induced by drought. *Span J Agric Res* 12:763–771
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930
- Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. *Front Microbiol* 7:332. doi:10.3389/fmicb.2016.00332
- Goh C, Vallejos DFV, Nicotra AB, Mathesius U (2013) The impact of beneficial plant-associated microbes on plant phenotypic plasticity. *J Chem Ecol* 39:826–839
- Hashem A, Abd-Allah EF, Alqarawi AA, Al Huqail AA, Egamberdieva D, Wirth S (2016) Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. *Saudi J Biol Sci* 23:272–281
- Jajic I, Sarna T, Strzalka K (2015) Senescence, stress, and reactive oxygen species. *Plants* 4:393–411
- Jogawat A, Saha S, Bakshi M, Dayaman V, Kumar M, Dua M, Varma A et al (2013) *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *Plant Signal Behav* 9:e26891. doi:10.4161/psb.26891
- Johnson JM, Alex T, Oelmüller R (2014) *Piriformospora indica*: the versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants. *J Tropical Agri* 52:103–122
- Kachroo A, Robin GP (2013) Systemic signaling during plant defense. *Curr Opin Plant Biol* 16:527–533
- Kadota Y, Shirasu K, Zipfel C (2015) Regulation of the NADPH oxidase RBOHD during plant immunity. *Plant Cell Physiol* 56:1472–1480. doi:10.1093/pcp/pcv063
- Kiirika LM, Schmitz U, Colditz F (2014) The alternative *Medicago truncatula* defense proteome of ROS-defective transgenic roots during early microbial infection. *Front Plant Sci* 5:341. doi:10.3389/fpls.2014.00341
- Kumar M, Yadav V, Tuteja N, Johri AK (2009) Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. *Microbiology* 155:780–790
- Lahrmann U, Ding Y, Banhara A, Rath M, Hajirezaei MR, Döhlemann S, von Wirén N, Parniske M, Zuccaro A (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. *Proc Natl Acad Sci U S A* 110:13965–13970
- Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmueller R, Varma A (2004) Phosphorus solubilizing symbiotic fungus *Piriformospora indica*. *Endocytobiosis Cell Res* 15:579–600
- Mine A, Sato M, Tsuda K (2014) Toward a systems understanding of plant–microbe interactions. *Front Plant Sci* 5:423. doi:10.3389/fpls.2014.00423
- Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Breusegem FV (2011) ROS signaling: the new wave? *Trends Plant Sci* 16 (6):300–309. doi:10.1016/j.tplants.2011.03.007

- Muthukumar T, Udaiyan K (2010) Growth response and nutrient utilization of *Casuarina equisetifolia* seedlings inoculated with bioinoculants under tropical nursery conditions. *New For* 40:101–118
- Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora indica* interaction under stress condition. *Front Plant Sci* 7:1574. doi:[10.3389/fpls.2016.01574](https://doi.org/10.3389/fpls.2016.01574)
- Neill SJ, Desikan R, Hancock J (2002) Hydrogen peroxide signaling. *Curr Opin Plant Biol* 5:388–395
- Oldroyd EDG, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. *Annu Rev Genet* 45:119–144
- Petersen LN, Ingle RA, Knight MR, Denby KJ (2009) OXI1 protein kinase is required for plant immunity against *Pseudomonas syringae* in *Arabidopsis*. *J Exp Bot* 60:3727–3735
- Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. *Agron Sustain Dev* 32:181–200
- Pozo MJ, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. *Curr Opin Plant Biol* 10:393–398
- Prasad R, Kamal S, Sharma PK, Oelmueller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plants morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. *J Basic Microbiol* 53:1016–1024
- Puppo A, Pauly N, Bosdari A, Mandon K, Brouquisse R (2013) Hydrogen peroxide and nitric oxide: key regulators of the legume Rhizobium and mycorrhizal symbioses. *Antioxid Redox Signal* 18:2202–2219
- Rasool S, Ahmad A, Siddiqi TO, Ahmad P (2013) Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiol Plant* 35:1039–1050
- Rentel MC, Lecourieux D, Ouaked F, Usher SL, Petersen L, Okamoto H et al (2004) OXI1 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis*. *Nature* 427:858–861
- Sarkar J, Ray A, Chakraborty B, Chakraborty U (2016) Antioxidative changes in *Citrus reticulata* L. induced by drought stress and its effect on root colonization by arbuscular mycorrhizal fungi. *Eur J Biol Res* 6:1–13
- Schouteden N, De Waele D, Panis B, Vos CM (2015) Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: a review of the mechanisms involved. *Front Microbiol* 6:1280. doi:[10.3389/fmicb.2015.01280](https://doi.org/10.3389/fmicb.2015.01280)
- Serfling A, Wirsel SGR, Lind V, Deising HB (2007) Performance of the biocontrol fungus *Piriformospora indica* on wheat under greenhouse and field conditions. *Phytopathology* 97:523–531
- Sewelam N, Kazan K, Schenk PM (2016) Global plant stress signaling: reactive oxygen species at the cross-road. *Front Plant Sci* 7:187. doi:[10.3389/fpls.2016.00187](https://doi.org/10.3389/fpls.2016.00187)
- Sherameti I, Tripathi S, Varma A, Oelmuller R (2008) The root colonizing endophyte *Piriformospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. *Mol Plant Microbe Interact* 21:799–807
- Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annu Rev Plant Biol* 62:227–250
- Song F, Li J, Fan X, Zhang Q, Chang W, Yang F, Geng G (2016) Transcriptome analysis of *Glomus mosseae/Medicago sativa* mycorrhiza on atrazine stress. *Sci Rep* 6:20245. doi:[10.1038/srep20245](https://doi.org/10.1038/srep20245)
- Suzuki N, Miller G, Salazar C, Mondal HA, Shulaev E, Cortes DF et al (2013) Temporal-spatial interaction between ROS and ABA controls rapid systemic acclimation in plants. *Plant Cell* 25:3553–3569
- Tahat MM, Sijam K (2012) Mycorrhizal fungi and abiotic environmental conditions relationship. *Res J Environ Sci* 6:125–133
- Tanaka A, Christensen MJ, Takemoto D, Park P, Scott B (2006) Reactive oxygen species play a role in regulating a fungus-perennial ryegrass mutualistic interaction. *Plant Cell* 18:1052–1066

- Trivedi D, Srivastava A, Verma PK, Tuteja N, Gill SS (2016) *Piriformospora indica*: a friend in need is a friend in deed. *Res Rev: J Botanical Sci* 5:16–19
- Vadassery J, Ranf S, Drzewiecki C, Mithöfer A, Mazars C, Scheel D et al (2009a) A cell wall extract from *Piriformospora indica* promotes growth of *Arabidopsis* seedlings and induces intracellular calcium elevation in roots. *Plant J* 59:193–206
- Vadassery J, Tripathi S, Prasad R, Varma A, Oelmüller R (2009b) Monodehydroascorbate reductase 2 and dehydroascorbate reductase 5 are crucial for a mutualistic interaction between *Piriformospora indica* and *Arabidopsis*. *J Plant Physiol* 166:1263–1274
- Vahabi K, Sherameti I, Bakshi M, Mrozinska A, Ludwig A, Reichelt M et al (2015) The interaction of *Arabidopsis* with *Piriformospora indica* shifts from initial transient stress induced by fungus-released chemical mediators to a mutualistic interaction after physical contact of the two symbionts. *BMC Plant Biol* 15:58. doi:10.1186/s12870-015-0419-3
- Varma A, Sherameti I, Tripathi S, Prasad R et al (2012) The symbiotic fungus *Piriformospora indica*: review. In: Hock B (ed) *Fungal associations, the mycota IX*, 2nd edn. Springer, Berlin, pp 231–254
- Verma S, Varma A, Rexer KH, Hassel A, Kost G, Sarbhoy A, Bisen P, Buthorn B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia* 90:896–902
- Vos C, Schouteden N, van Tuinen D, Chatagnier O, Elsen A, De Waele D et al (2013) Mycorrhiza-induced resistance against the root-knot nematode *Meloidogyne incognita* involves priming of defense gene responses in tomato. *Soil Biol Biochem* 60:45–54. doi:10.1016/j.soilbio.2013.01.013
- Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M et al (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc Natl Acad Sci U S A* 102:13386–13391
- Wu Q, Zou Y, Abd-Allah EF (2014) Mycorrhizal association and ROS in plants. In: Ahmad P (ed) *Oxidative damage to plants*, Chap. 15. Academic Press, San Diego, pp 453–475. doi:10.1016/B978-0-12-799963-0.00015-0
- Xia XJ, Wang YJ, Zhou YH, Tao Y, Mao WH, Shi K et al (2009) Reactive oxygen species are involved in brassinosteroid-induced stress tolerance in cucumber. *Plant Physiol* 150:801–814
- Xu E, Brosche M (2014) Salicylic acid signaling inhibits apoplastic reactive oxygen species signaling. *BMC Plant Biol* 14:155. doi:10.1186/1471-2229-14-155
- Yan J, Tsuichihara N, Etoh T, Iwai S (2007) Reactive oxygen species and nitric oxide are involved in ABA inhibition of stomatal opening. *Plant Cell Environ* 30:1320–1325
- Ye W, Shen CH, Lin Y, Chen PJ, Xu X, Oelmüller R, Yeh KW, Lai Z (2014) Growth promotion-related miRNAs in oncidium orchid roots colonized by the endophytic fungus *Piriformospora indica*. *PLoS One* 9(1):e84920. doi:10.1371/journal.pone.0084920

Chapter 13

Stimulated Growth of *Lycopersicum esculentum* CLA 1131 in Presence of *Piriformospora indica* and Vermicompost

Reshma Tuladhar, Kenneth Shahi, Sujen Man Shrestha, Anjana Singh, and Ajit Varma

Abstract In the mutualistic association between plant and mycorrhiza, plant benefits by gaining an improved nutrient and water acquisition through fungal hyphae and/or an enhanced abiotic stress tolerance. Since mycorrhiza facilitates the plant in the nutrient uptake from the soil, fertility of soil is necessary for the availability of the essential nutrients. The axenically cultivable root-colonizing endophytic fungi *Piriformospora indica* treated tomato plant (*Lycopersicum esculentum* CLA 1131); when supplemented with vermicompost, the growth and biomass were enhanced. Mycorrhizal colonization was improved in the presence of vermicompost. The amount of essential nutrient nitrogen, phosphorous, and potassium content in plant was improved by the colonization with *P. indica* and influenced by the nutrient conditions in the soil. The efficiency of nutrient uptake by *P. indica* is complemented by vermicompost.

13.1 Introduction

The increase in crop production adopting environment-friendly strategy is necessary to address the demand of food for growing human population and need of sustainable agriculture. The green revolution, which was active during the 1940s and 1960s, supported the use of chemical fertilizers and pesticides to increase the yield (Tilman 1998). However, the negative impact of these chemicals in human health and environment has necessitated the use of alternative solutions to increase

R. Tuladhar • K. Shahi • A. Singh (✉)

Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
e-mail: anjanas67@gmail.com

S.M. Shrestha

Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal

A. Varma

Amity Institute of Microbial Technology, Amity University, Sector 125, Noida 201303, Uttar Pradesh, India

crop yields sustainably. The application of biological solution, which includes the manipulation and exploitation of beneficial plant-microbe interactions, is the sustainable approach.

The consortium of plant-microbe interaction is highly complex, comprising diverse microbial species. The complex relationship based on mutual interaction between diverse microbial population and plants proliferates in the rhizosphere and within the plant itself (Evangelisti et al. 2014). The root fungus mycorrhiza is an intriguing component which develops within the rhizosphere and associates symbiotically promoting the growth and health of the plant (Malla et al. 2002; Goltapeh et al. 2008). The diverse mycorrhiza includes ectomycorrhiza, endomycorrhiza, ectendomycorrhiza, and arbuscular mycorrhizal fungi (AMF) (Gosal et al. 2013). AMF occur on a vast taxonomic range of plants and hence most commonly reported group (Malla et al. 2002). The multitude benefits from arbuscular mycorrhizal association improved nutrient uptake, mineralization of organic nutrients, resistance to abiotic and biotic stress, etc. (Cruz et al. 2013; Prasad et al. 2015; Gill et al. 2016). Despite the plethora of benefits, AMF use is limited in agriculture owing to its difficulty in producing inoculums (DeClerk et al. 2005). It is obligate and thrives only on the living cells of the host plant.

However, *Piriformospora indica*, a non-obligate biotroph discovered by Varma and his co-worker (Verma et al. 1998), has potential for agricultural application for its ability to grow in synthetic medium. This axenically cultivable arbuscular mycorrhiza-like fungus endophyte similar to AMF in many aspects (Varma and Schuepp 1994; Varma et al. 1999; Singh et al. 2000; Rai and Varma 2005; Prasad et al. 2005) establishes mutualistic interactions with a broad variety of plant species (Jacobs et al. 2013). It has the ability to colonize the roots of wide host range from medicinal plants and ornamental plants to economically important crops (Singh et al. 2000; Prasad et al. 2008a, b, 2013). The host plants benefited by the colonization of this endophyte include crops like wheat, maize, and sugarcane (Rai et al. 2001; Waller et al. 2005; Baltruschat et al. 2008); legume crops like soybean, pea, and bean; and several medicinal plants (Kumari et al. 2004; Oelmüller et al. 2009; Das et al. 2013; Bagde et al. 2010, 2014).

Root colonized by *P. indica* provides various benefits to host plant which include growth promotion and enhancement for better biomass and yield (Gosal et al. 2013) and tolerance to abiotic stress (Bagyaraj and Varma 1995) and limit severity of plant disease (Fakhro et al. 2010). *P. indica* promote the plant with its ability to extract, mobilize, and transport phosphorous as well as several micronutrients from soil (Gosal et al. 2013). The fungus possesses positive phyto-promotional effects due to plant bio-regulation ability, apart from its role in mobilization and transportation of the plant unavailable phosphorous in soil (Gosal et al. 2013; Malla et al. 2004). The plant physiology is stimulated increasing vegetative growth, inducing resistance against plant pathogens, and increasing yield.

The effect of the interaction, however, depends on various factors such as amount of inoculums, the time point of inoculation, and nutrient content in the environment (Andrade-Linares et al. 2013). When high amounts of *P. indica* were inoculated in soil under nutrient-limiting conditions of low amounts of nitrogen and phosphorous, negative effect was observed in tomato plant (Andrade-Linares et al. 2013). Tomato is one of the most consumed vegetable crops worldwide. Researchers have demonstrated that growth of tomato plant is improved by colonization of its root with *P. indica* (Fakhro et al. 2010; Andrade-Linares et al. 2013). In this chapter, we review the impact of dual inoculation of endophyte *P. indica* and vermicompost on the growth of tomato plant.

13.2 Effect of Dual Inoculation on Vegetative Growth

Vermicompost is produced by earthworms in the form of worm cast upon feeding on biodegradable materials. This product of biodegradation of organic materials through interactions between earthworms and microorganisms (Sallaku et al. 2009) is rich in nitrogen, phosphorous, and potassium (NPK).

Tomato plant (*Lycopersicum esculentum* CLA 1131) inoculated with *P. indica* and grown in soil supplemented with vermicompost increased the length and dry weight of shoot and root compared to the tomato plant treated with *P. indica* or vermicompost alone (Fig. 13.1). The experiment was carried out in earthen pot filled with soil supplemented with vermicompost. The leaf number per plant was highest in the plant grown in presence of both *P. indica* and vermicompost in soil (Fig. 13.2). Increased biomass of the leaves by up to 20% has been observed in tomato plants colonized by *P. indica* (Fakhro et al. 2010). The length and dry weight of root and shoot were measured following 90 and 120 days of transplantation. The growth of plant in terms of root and shoot parameters was highest in *P. indica*-inoculated plant supplemented with vermicompost compared to plant with single treatment of *P. indica* alone or vermicompost alone (Figs. 13.3 and 13.4).

Singh et al. (2001) and Malla et al. (2002) reported significant increment in shoot length when *S. calva* and *W. somnifera* were inoculated with *P. indica*. The promotion of early growth stages of plant is owed to accelerated root development, and age-dependent regulation of genes shifted to earlier time points in *P. indica*-colonized roots (Waller et al. 2005, 2008). In addition, *P. indica* promote plant growth by inhibiting the ethylene signaling which impedes the plant development (Barazani et al. 2005).

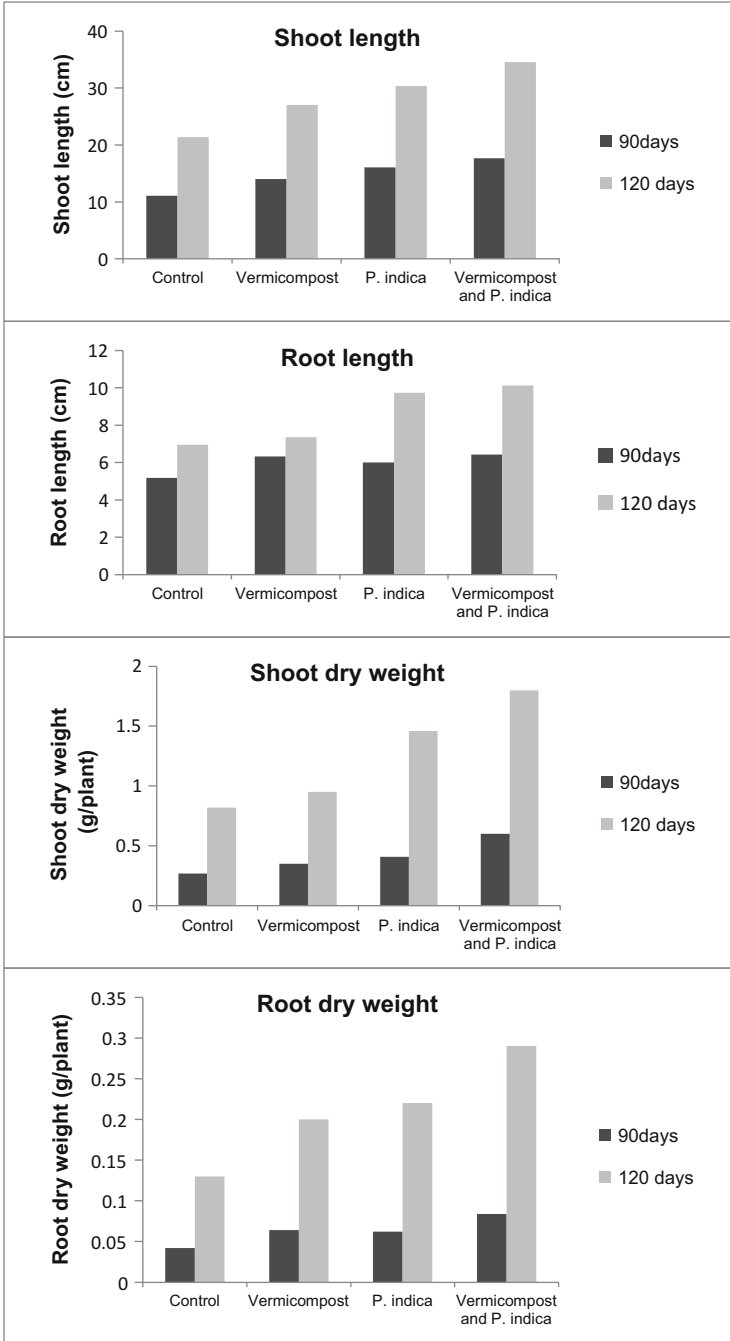


Fig. 13.1 Impact of *P. indica* and vermicompost on vegetative growth on 90 and 120 days after inoculation. Tomato plants (*Lycopersicon esculentum* CLA 1131) in pot culture were inoculated with vermicompost only, *P. indica* alone, and dual inoculation of *P. indica* and vermicompost in three consecutive experiments. The plants harvested after 90 and 120 days of plantation were measured for root and shoot length and dry weight of root and shoot

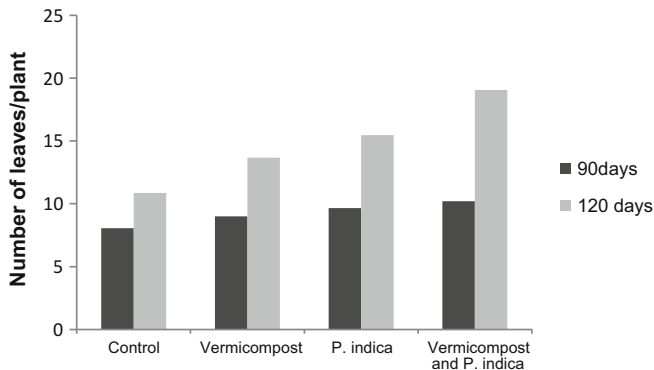


Fig. 13.2 Impact of *P. indica* and vermicompost on the leaf number. Tomato plants (*Lycopersicum esculentum* CLA 1131) inoculated with vermicompost only, *P. indica* alone, and dual inoculation of *P. indica* and vermicompost were measured for the average number of leaves per plant after 90 and 120 days of plantation

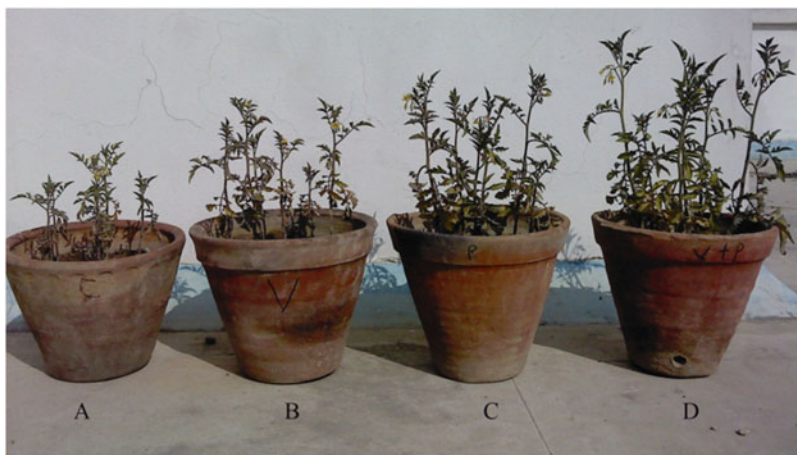


Fig. 13.3 Effect of dual inoculation of *P. indica* and vermicompost on the growth of tomato plant (*Lycopersicum esculentum* CLA 1131) after 90 days of transplantation. A (control), B (vermicompost), C (*P. indica*), and D (*P. indica* and vermicompost)

13.3 Mycorrhizal Colonization in Presence of Vermicompost

The abundance of mycorrhizal fungi in soil has been indicated by the measurement of the extent to which the roots are colonized with mycorrhizae (Hayman and Stovold 1979; Sparling and Tinker 1978). The active symbiotic phase is reflected from the mycorrhizal root colonization. Dual inoculation of tomato plant with

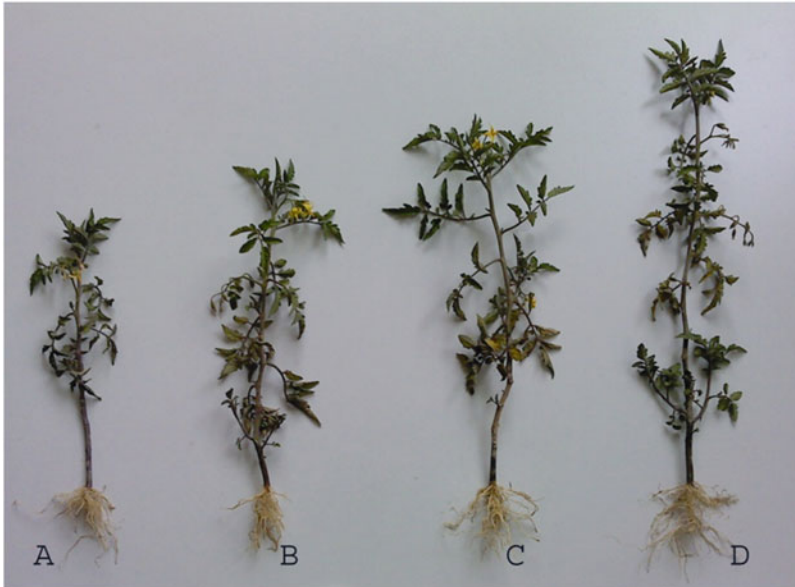


Fig. 13.4 Effect of dual inoculation of *P. indica* and vermicompost on tomato plant (*Lycopersicum esculentum* CLA 1131) after 120 days of transplantation. A (control), B (vermicompost), C (*P. indica*), and D (*P. indica* and vermicompost)

P. indica and vermicompost improved mycorrhizal colonization compared to *P. indica* alone, while colonization was absent in control plant (Fig. 13.5). Vermicompost improves texture and properties of soil (Edwards and Burrows 1988) making it conducive for soil microflora. Mycorrhizal colonization has been significantly increased in presence of vermicompost (Kale et al. 1992). The chlamydospores colonized in the root of tomato plant in presence of vermicompost are greater than in absence of vermicompost (Fig. 13.6). This suggests that nutrient-rich soil facilitates the colonization of *P. indica* which in turn will provide enhanced benefit to the plant.

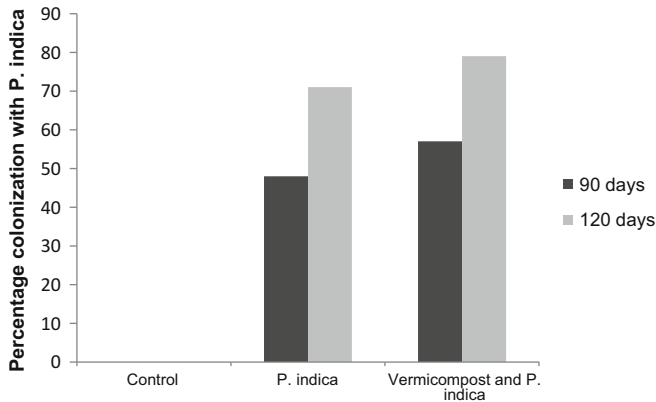


Fig. 13.5 Mycorrhizal colonization percentage in the root of tomato plants (*Lycopersicum esculentum* CLA 1131) treated with *P. indica* alone and dual inoculation of *P. indica* and vermicompost

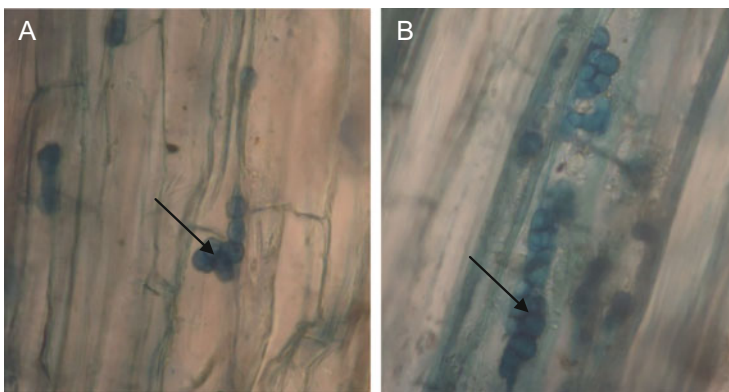


Fig. 13.6 Tomato root colonized with *P. indica*. The roots of tomato plant in pot culture inoculated with *P. indica* alone and dual inoculation with *P. indica* and vermicompost were harvested and stained with trypan blue. (a) Chlamydozoospores in root of tomato plant inoculated with *P. indica* alone and (b) chlamydozoospores in dual inoculation with *P. indica* and vermicompost. Arrow indicates chlamydozoospores

13.4 Effect on Nitrogen, Phosphorous, and Potassium Content in the Plant

Organic fertilizers are known to contain nitrogen (N), phosphorous (P), and potassium (K), the essential macronutrients required for the growth and development of plant. Vermicompost, a superior organic manure, increases the soil fertility for its high percentage of NPK and water retention ability (Edwards and Burrows 1988; Acharya 1997) enhances biomass production of number of crops (Hidalgo 1999). Colonization of plant roots with *P. indica* increases the efficiency in the uptake of

these macronutrients by plant, aiding in the optimal growth of plant as well as ensuring the maximum utilization of the vermicompost.

Apart from its serving as an important building block of amino acids, nucleic acids, and chlorophyll in plant, nitrogen is an essential regulator in carbon and amino acid metabolism (Frink et al. 1999; Cai et al. 2012). Plants absorb N from soil in the form of nitrate and ammonia/ammonium (Kulcheski et al. 2015). Legumes are benefitted through the microbial symbiosis in acquiring nitrogen. The use of nitrogenous fertilizers is in practice for the plants that do not exhibit microbial symbiosis, but it has contributed to serious problems of soil and water pollution. Major portion of the nitrogenous fertilizers are lost due to incomplete capture by plant or through conversion to nitrous oxide (Montzka et al. 2011). Thus, the efficient uptake of N by plants is necessary to be established.

Mycorrhizal association is the best alternative for plants that do not symbiotically associate with N-fixing bacteria for the acquisition of N from soil. The mycorrhizal mycelium has the ability to transport organic and inorganic N sources from soil and export to the plant (Bonfante and Genre 2010). Symbiotic association with AMF and *P. indica* was found to improve the N acquisition by the plants, of which *P. indica* was more efficient (Cruz et al. 2013). The difference in their N uptake is that *P. indica* mediates nitrate uptake from soil, while AMF preferentially absorb ammonium (Gosal et al. 2013).

In most soil, large portion of P is unavailable to plant since they are immobilized (Marschner 1995). The soluble form is orthophosphate which is very low in concentration in soil. To acquire P under limiting condition, plant either explores for available P by extending root and extensively branching it off or enhances secretion of phosphatase and expression of new kind of Pi transporter in root cell (Johri et al. 2015).

P. indica is reported to enhance plant growth rate through an increase in nutrient uptake, especially P that is relatively immobile in soils (Singh et al. 2001; Varma et al. 2001). *P. indica* inoculation could have also induced soybean to absorb more nutrients by increasing the absorbing surface area. Enhanced activity of acid phosphatase and alkaline phosphatase was noticed in the rhizosphere soil of rice plants inoculated with *P. indica* (Das et al. 2014). A high-affinity phosphate transporter PiPT is present in *P. indica* which improve Pi nutrition levels in the host plant under P-limiting conditions (Johri et al. 2015). *P. indica* mediate more efficient phosphate (Pi) uptake by plant independent to the degree of root colonization suggesting it to be an alternative to other mycorrhizal fungi (Johri et al. 2015).

Potassium is essential for plant development and reproduction, yield, and responses to abiotic stress (Demidchik et al. 2014; Zorb et al. 2014; Zhang et al. 2015). Vermicompost enhances in the content of the essential nutrient in the soil as it led to significant increase in soil enzyme activities such as urease, phosphomonoesterase, and phosphodiesterase (Albiach et al. 2000). Besides, plant growth-promoting bacteria stimulate solubilization of nutrients (Rodriguez and Fraga 1999) and production of growth hormones (Correa et al. 2004). Increased nitrogen, phosphorous, and potassium content in plants has been resulted from the association of *P. indica* with plant roots. Higher NPK content in root and shoot of chick pea

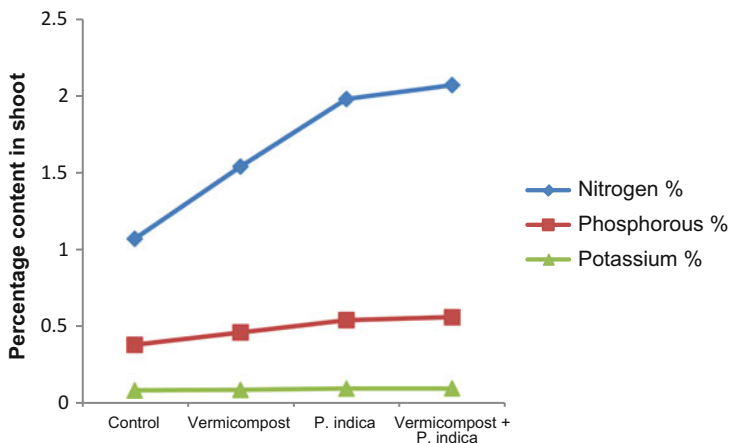


Fig. 13.7 Effect of dual inoculation of *P. indica* and vermicompost on NPK content of shoot. Percentage of nitrogen (diamond marked), phosphorus (square marked), and potassium (triangular marked) present in the shoots of tomato plants after 120 days of transplantation

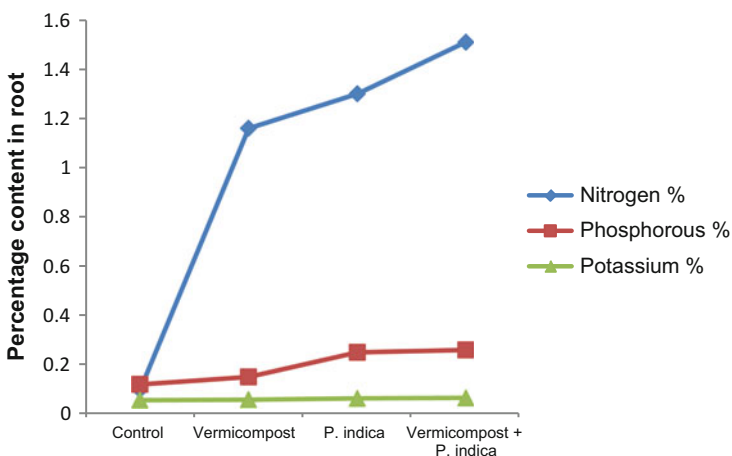


Fig. 13.8 Effect of dual inoculation of *P. indica* and vermicompost on NPK content of shoot. Percentage of nitrogen (diamond marked), phosphorus (square marked), and potassium (triangular marked) present in the roots of tomato plants after 120 days of transplantation

and black lentil plants was established when inoculated with *P. indica* (Kumar et al. 2012; Nautiyal et al. 2010). The increased NPK in *Phaseolus* was associated with inoculation of *P. indica* and *Rhizobium* in presence of vermicompost (Tuladhar et al. 2013).

The association of *P. indica* in presence of vermicompost in soil has increased the NPK content in the tomato plant. Compared to phosphorous and potassium, the nitrogen uptake has been hugely elevated (Figs. 13.7 and 13.8). The P uptake is more efficient in soybean through tripartite association of *P. indica*, *Rhizobium*, and

vermicompost (Tuladhar et al. 2013) compared to dual effect of *P. indica* and vermicompost on tomato plant (Figs. 13.7 and 13.8), while the effect on N and K uptake is alike. It seems various factors are involved in the process of nutrient uptake leading to variation in the effect of mycorrhiza on the diverse plant species.

13.5 Conclusion

As in the circumstance of numerous types of plants, the vegetative growth of tomato plant is accelerated by *P. indica*. However, there are several conditions essential for the efficiency in the nutrient uptake. Supplementing vermicompost to enrich the soil has increased the efficiency of *P. indica* to promote the growth of tomato plant. Such effect may not be identical to every variety of plant and under different environmental conditions. It is necessary to identify the optimal conditions to augment the effectiveness of this mycorrhiza.

Acknowledgments Ajit Varma is thankful to DBT for partial funding and DST for providing confocal microscope.

References

- Acharya MS (1997) Integrated vermiculture for rural development. *Int J Rural Stud* 4:8–10
- Albiach R, Canet R, Pomares F, Ingelmo F (2000) Microbial biomass content and enzymatic and activities after application of organic amendments to a horticultural soil. *Bioresour Technol* 75:43–48
- Andrade-Linares DR, Muller A, Fakhro A, Schwarz D, Franken P (2013) Impact of *Piriformospora indica* on Tomato. In: Varma A et al (eds) *Piriformospora indica*. Soil biology, vol 33. Springer, Heidelberg, pp 107–117
- Bagde US, Prasad R, Varma A (2010) Interaction of *Piriformospora indica* with medicinal plants and of economic importance. *Afr J Biotechnol* 9:9214–9226
- Bagde US, Prasad R, Varma A (2014) Impact of culture filtrate of *Piriformospora indica* on biomass and biosynthesis of active ingredient aristolochic acid in *Aristolochia elegans* Mart. *Int J Biol* 1:29–37
- Bagyaraj DJ, Varma A (1995) Interaction between arbuscular mycorrhizal fungi and plants and their importance in sustainable agriculture in arid and semi-arid tropics. *Adv Microb Ecol* 14:119–142
- Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel KH, Schäfer P, Schwarczinger I, Zuccaro A, Skoczowski A (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytol* 180:501–510
- Barazani O, Benderoth M, Groten K, Kuhlemeier C, Baldwin IT (2005) *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in *Nicotiana attenuate*. *Oecologia* 146:234–243
- Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nat Commun* 1:1–11

- Cai H, Lu Y, Xie W, Zhu T, Lian X (2012) Transcriptome response to nitrogen starvation in rice. *J Biosci* 37:731–747
- Correa JD, Barrios ML, Galdona RP (2004) Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. *Plant and Soil* 266:75–84
- Cruz C, Fegghi Z, Martins-Loucao MA, Varma A (2013) Plant nitrogen use efficiency may be improved through symbiosis with *Piriformospora indica*. In: Varma A et al (eds) *Piriformospora indica*. Soil biology, vol 33. Springer, Heidelberg, pp 285–293
- Das A, Prasad R, Srivastava RB, Deshmukh S, Rai MK, Varma A (2013) Co-cultivation of plants with medicinal plants: case studies. In: Varma A, Kost G, Oelmüller R (eds) *Piriformospora indica*: sebacinales and their biotechnological applications. Springer, Heidelberg, pp 149–171
- Das J, Ramesh KV, Maithri U, Mutangana D, Suresh CK (2014) Response of aerobic rice to *Piriformospora indica*. *Indian J Exp Biol* 52:237–251
- DeClerk S, Strullu DG, Fortin JA (2005) In vitro culture of Mycorrhiza. Springer, New York
- Demidchik V, Straltsova D, Medvedev SS, Pozhvanov GA, Sokolik A, Yurin V (2014) Stress-induced electrolyte leakage: the role of K⁺-permeable channels and involvement in programmed cell death and metabolic adjustment. *J Exp Bot* 65:1259–1270
- Edwards CA, Burrows I (1988) The potential of earthworm compost as plant growth media. In: Neuhauser E, Edwards CA (eds) *Earthworms in waste and environmental management*. SPB Academic, The Hague
- Evangelisti E, Rey T, Schornack S (2014) Cross-interference of plant development and plant-microbe interactions. *Curr Opin Plant Biol* 20:118–126
- Fakhro A, Andrade-Linares DR, von Barga S, Bandte M, Büttner C, Grosch R, Schwarz D, Franken P (2010) Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza* 20:191–200
- Frink CR, Waggoner PE, Ausubel JH (1999) Nitrogen fertilizer: retrospect and prospect. *Proc Natl Acad Sci U S A* 96:1175–1180
- Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. *Front Microbiol* 7:332. doi:10.3389/fmicb.2016.00332
- Goltapeh EM, Danesh YR, Prasad R, Varma A (2008) Mycorrhizal fungi: what we know and what should we know? In: Varma A (ed) *Mycorrhiza*, 3rd edn. Springer, Berlin, pp 3–27
- Gosal SK, Kalia A, Varma A (2013) *Piriformospora indica*: perspectives and retrospectives. In: Varma A et al (eds) *Piriformospora indica*. Soil biology, vol 33. Springer, Berlin, pp 53–77
- Hayman DS, Stovold GE (1979) Plant growth response to vesicular arbuscular mycorrhiza. *New Phytol* 71:41–47
- Hidalgo P (1999) Earthworm castings increase germination rate and seedling development of cucumber. *Miss Agric For Exp Station Res Rep* 22:135–142
- Jacobs S, Kogel K, Schafer P (2013) Root-based innate immunity and its suppression by the mutualistic fungus *Piriformospora indica*. In: Varma A et al (eds) *Piriformospora indica*. Soil biology, vol 33. Springer, Berlin pp 223–237
- Johri AK, Oelmüller R, Dua M, Yadav V, Kumar M, Tuteja N, Varma A, Bonfante P, Persson BL, Stroud RM (2015) Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front Microbiol* 6:1–13
- Kale RD, Mallesh BC, Bano K, Bagyaraj DJ (1992) Influence of vermicompost applications on the available macronutrients and selected microbial populations in a paddy field. *Soil Biol Biochem* 24:700–702
- Kulcheski FR, Côrrea R, Gomes IA, de Lima JC, Margis R (2015) NPK macronutrients and microRNA homeostasis. *Front Plant Sci* 6:1–19
- Kumar V, Sarma MVRK, Saharan K, Srivastava R, Kumar L, Sahai V, Bisaria VS, Sharma AK (2012) Effect of formulated root endophytic fungus *Piriformospora indica* and plant growth promoting rhizobacteria fluorescent pseudomonads R62 and R81 on *Vigna mungo*. *World J Microbial Biotechnol* 28:595–603

- Kumari R, Pham GH, Prasad R, Sachdev M, Srivastava A, Yadav V, Verma PK, Sharma S, Malla R, Singh A, Mayura AK, Prakash S, Pareek A, Rexer KH, Kost G, Garg AP, Oelmüller R, Sharma MC, Varma A (2004) *Piriformospora indica*: fungus of the millennium. In: Podila G, Varma A (eds) Basic research and applications: mycorrhizae. IK International-India/Kluwer Academic, New York, pp 259–295
- Malla R, Singh A, Md Z, Yadav V, Suniti, Verma A, Rai M, Varma A (2002) *Piriformospora indica* and plant growth promoting rhizobacteria: an appraisal. In: Rao GP, Bhat DJ, Lakhanpal TN, Manoharichari C (eds) Frontiers of fungal diversity in India. pp 401–419
- Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmueller R, Varma A (2004) Phosphorus solubilizing symbiotic fungus *Piriformospora indica*. Endocytobiosis Cell Res 15:579–600
- Marschner H (1995) Nutrient availability in soils: mineral nutrition of higher plants, 2nd edn. Academic Press, London, pp 483–507
- Montzka SA, Dlugokencky EJ, Butler JH (2011) Non-CO₂ greenhouse gases and climate change. Nature 476:43–50
- Nautiyal CS, Chauhan PS, Gupta SMD, Seem K, Varma A, Staddon WJ (2010) Tripartite interactions among *Paenibacillus lentimorbus* NRRL B-30488, *Piriformospora indica* DSM 11827 and *Cicer arietinum* L. World J Microbial Biotechnol 26:1393–2399
- Oelmüller R, Sherameti I, Tripathi S, Varma A (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis 49:1–17
- Prasad R, Pham GH, Kumari R, Singh A, Yadav V, Sachdev M, PeskanT HS, Oelmuller R, Garg AP, Varma A (2005) Sebacinaceae: culturable mycorrhiza-like endosymbiotic fungi and their interaction with non-transformed and transformed roots. In: Declerck S, Strullu DG, Fortin JA (eds) In vitro culture of mycorrhizas, vol 4. Springer, Berlin, pp 291–312
- Prasad R, Bagde US, Pushpangdan P, Varma A (2008a) *Bacopa monniera* L.: pharmacological aspects and case study involving *Piriformospora indica*. Int J Integr Biol 3:100–110
- Prasad R, Sharma M, Kamal S, Rai MK, Rawat AKS, Pushpangdan P, Varma A (2008b) Interaction of *Piriformospora indica* with medicinal plants. In: Varma A (ed) Mycorrhiza. Springer, Berlin, pp 655–678
- Prasad R, Kamal S, Sharma PK, Oelmueller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plants morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. J Basic Microbiol 53:1016–1024
- Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) Plant growth-promoting rhizobacteria and medicinal plants. Springer International Publishing, Berlin, pp 247–260
- Rai M, Varma A (2005) Arbuscular mycorrhiza-like biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica* Nees. Electron J Biotechnol 8:107–110
- Rai MK, Singh A, Arya D, Varma A (2001) Positive growth responses of *Withania somnifera* and *Spilanthes calva* cultivated with *Piriformospora indica* in field. Mycorrhiza 11:123–128
- Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
- Sallaku G, Babaj I, Kaciu S, Balliu A (2009) The influence of vermicompost on plant growth characteristics of cucumber (*Cucumis sativus* L.) seedlings under saline conditions. J Food Agric Environ 7:869–872
- Singh A, Sharma J, Rexer KH, Varma A (2000) Plant productivity determinants beyond minerals, water and light. *Piriformospora indica*: a revolutionary plant growth promoting fungus. Curr Sci 79:101–106
- Singh A, Singh A, Rexer KH, Kost G, Varma A (2001) Root endosymbiont: *Piriformospora indica*—a boon for orchids. J Orchid Soc India 15:89–101
- Sparling GP, Tinker PB (1978) Mycorrhizal in pennine grassland. J Appl Ecol 15:943–950
- Tilman D (1998) The greening of the green revolution. Nature 396:211–212
- Tuladhar R, Shrestha J, Singh A, Varma A (2013) Enhanced productivity associated with tripartite symbiosis between *Phaseolus*, Rhizobia, and *Piriformospora indica*: in presence of

- vermicompost. In: Varma A et al (eds) *Piriformospora indica*. Soil biology, vol 33. Springer, Berlin, pp 191–199
- Varma A, Schuepp H (1994) Positive influence of arbuscular mycorrhizal fungus on in vitro raised hortensia plantlets. *Angew Bot* 68:108–113
- Varma A, Verma S, Sudha SN, Britta B, Franken P (1999) *Piriformospora indica* – a cultivable plant growth promoting root endophyte with similarities to arbuscular mycorrhizal fungi. *Appl Environ Microbiol* 65:2741–2744
- Varma A, Singh A, Sudha SN, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T, Bleichert O, Rexer KH, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica*: a plant stimulator and pathogen inhibitor arbuscular mycorrhizae like fungus. In: Markandey DK, Markandey NR (eds) *Microorganisms in bioremediation*. Capital Book Company, New Delhi, pp 71–80
- Verma S, Varma A, Rexer KH, Kost G, Sarbhoy A, Bisen P, Buetehorn B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia* 90:896–902
- Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, von Wettstein D, Franken P, Kogel KH (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance and higher yield. *Proc Natl Acad Sci U S A* 102:13386–13391
- Waller F, Mukherjee K, Deshmukh SD, Achatz B, Sharma M, Schaefer P, Kogel KH (2008) Systemic and local modulation of plant responses by *Piriformospora indica* and related Sebaciales species. *J Plant Physiol* 165:60–70
- Zhang YM, Zhang HM, Liu ZH, Li HC, Guo XL, Li GL (2015) The wheat NHX antiporter gene TaNHX2 confers salt tolerance in transgenic alfalfa by increasing the retention capacity of intracellular potassium. *Plant Mol Biol* 87:317–327
- Zorb C, Senbayram M, Peiter E (2014) Potassium in agriculture-status and perspectives. *J Plant Physiol* 171:656–669

Chapter 14

Promotion and Value Addition to Some Important Medicinal Plants Under Saline Condition by Intervention of a Novel Mycorrhizal Formulation

Priyanka Sharma, Hemesh Joshi, Amit C. Kharkwal, Narendra Tuteja, and Ajit Varma

Abstract The use of plants in the remediation of saline and sodic soils is an emerging low cost approach in the reclamation of abandoned irrigated fields. The present study focuses on use of a novel AM-like-Fungus *Piriformospora indica* for phytoremediation by early intervention with selected medicinal plants. *Piriformospora indica*, a root endophytic fungus, has been reported to promote growth of many plants under normal condition and allow the plants to survive under stress conditions. The fungus is able to associate with the roots of various plant species in a manner similar to mycorrhiza and promotes plant growth. *P. indica* has been reported to induce resistance in the monocotyledonous plant barley to fungal diseases, along with tolerance to salt stress without affecting the plant productivity. The prospects for improved agriculture, by the use of microbial inoculants as biofertilizers or biological control agents, are particularly good in less intensive, low-input agricultural systems. Hence, in developing countries microbial inoculation of plants could be of great importance. The advantages are: better yields, lower costs, reduced dependence on chemicals, and sustainable environment. The production of microbial inoculants is not very difficult; unsophisticated fermentors of modest volume can be used to produce significant quantities of bioinoculants. Present study was undertaken to investigate the effect of consortium of *P. indica* and *Azotobacter chroococcum* on salinity stress tolerance of important medicinal plants. Both inoculated and non-inoculated plantlets were subjected to four levels of salinity treatment—0, 100, 200, and 300 mM NaCl. The salinity stress decreased the ability of the consortium to colonize roots of plants, but the interaction resulted in an overall increase in plant biomass and greater shoot and root length as well as number of shoots and roots. The inoculated plantlets had significantly higher secondary metabolite contents as determined using HPLC. The higher secondary

P. Sharma • H. Joshi • A.C. Kharkwal (✉) • N. Tuteja • A. Varma
Amity Institute of Microbial Technology, Amity University, Block E-3, 4th Floor, Sector 125,
Noida 201303, Uttar Pradesh, India
e-mail: ackharkwal@amity.edu

metabolite content may help the plants ameliorate oxidative stress resulting from high salinity. This was achieved by early interaction of the selected medicinal plants, namely, *Glycyrrhiza glabra*, *Aloe vera*, *Bacopa monnieri*, *Asparagus racemosus*, *Coleus forskohlii*, *Withania somnifera*, *Vinca rosea*, and *Ocimum* sp. with *P. indica* and *A. chroococcum* under in vitro (plant tissue culture), in vivo (greenhouse), and field conditions in Amity University and Issapur, respectively. For development of different formulations, *P. indica* was grown in large-scale fermentor. The formulated inoculum produced along with the culture filtrate was used for early intervention during the growth of plants. The plants were intervened both under in vitro (tissue culture) and ex vitro (greenhouse) conditions. The treated plants were then transferred to field for further evaluation at Issapur farm in Delhi. Plant growth was assessed on the basis of plant biomass and other morphological parameters. The rejuvenation of soil with the microbes also makes it suitable for organic cultivation of crops in the forthcoming years hence reducing the impact of chemical fertilizers.

14.1 Introduction

In nature, plants are frequently exposed to adverse environmental conditions. Soil salinity is one of the major constraint that drastically affects the growth, yield, and survival of the plants (Ashraf and Harris 2004; Jin et al. 2007). Currently, high soil salinity occupies 7% of Earth's land surface, and it is predicted that by the end of twenty first century, 50% of arable land will be affected (Evelin and Kapoor 2014). Salt affected lands occur in almost all climatic regions, ranging from humid tropics to the Polar Regions and from below sea level (around the Dead Sea) to mountains above 5000 m (Rocky Mountains) (Aggarwal et al. 2012). The presence of excess ions in the rhizosphere leads to lowering of soil water potential and limits the availability of water to the plant. This results in many detrimental effects such as growth reduction, injury of foliage, nutrient deficiencies, destruction of soil structure, cell death, and root necrosis, finally affecting the growth and survival of the plant (Aggarwal et al. 2012; Lozano 2003; Baltruschat et al. 2008). Soil salinity along with other abiotic stresses is responsible for more than 50% reduction in crop productivity. The common cations associated with soil salinity are Na^+ , Ca^{2+} , and Mg^{2+} , whereas the major anions are Cl^- , SO_4^{2-} , and HCO_3^- . However, the most damaging ions that deteriorate the soil structure and can be toxic to plants are Na^+ and Cl^- (Hasegawa et al. 2000). Soil salinization results due to weathering of minerals, improper agricultural management practices, poor water management, low precipitation, limited rainfall, high evaporation, and heavy irrigation (Chen et al. 2011). This increase in soil salinization is against the needs of expanding global population, which requires an increase of 20% food production in developed countries and 60% in developing countries over next 30 years (Galvani 2007).

14.2 Effect of Salinity on Plant Growth

Increase in soil salinity results in reduction of osmotic potential of soil solution, which make it necessary for the plant to maintain a lower intracellular osmotic potential. The response of all plants to decreased osmotic potential is turgor loss, which results in stomatal closure, followed by reduction in gas exchange (i.e., transpiration and photosynthesis). Hence, the major cause of growth inhibition under salt stress is decreased turgor pressure (Ashraf and Tufail 1994). The stomatal closure in turn decreases the availability of CO_2 and reduces photosynthesis, which ultimately lead to production of Reactive Oxygen Species (ROS). Also, lower availability of NADP to accept electrons from PSI results in reduction of O_2 with a concomitant generation of ROS (Sudhakar et al. 2001; Gill and Tuteja 2010). The total ionic concentration adversely affects the growth of the plants. With increase in salinity, the uptake and translocation of ions get reduced. It has been reported that the salinity significantly increases K^+ and Cl^- in the leaves and stems, while reduces Ca^{2+} , K^+ , Mg^{2+} , and NO_3^- . The K^+ and Ca^{2+} are required to maintain the selectivity and integrity of membrane. Thus, high Na^+/K^+ ratio alters the membrane selectivity and results in passive accumulation of Na^+ ion in the roots and shoots. It also affects other processes such as stomatal movement, photosynthesis, and transpiration (Hu and Schmidhalter 1997). Thus, plants need to adapt a strategy to protect themselves from such stresses.

14.3 Plant Microbe Interaction

Plant growth and development is synergistic combination of a number of environmental factors. Plants are being attacked by a number of microorganisms, with an aim to acquire nutrients from them. The consequences of the interactions can be harmful (as in parasitism), neutral, and beneficial (as in mutualism) (Shen et al. 2006; Thrall et al. 2007). The plants are in association with a number of microbes, including soil microbes, atmospheric microbes, plant colonizing microbes, plant surface microbes, and internal microbes. Most of the microbes act synergistically with plants and do not cause any ill effects to them. However, selection of appropriate microbe is important that can develop positive interaction with the plant and boost plant growth and productivity and can improve the soil health. Many of such microbes establish themselves in the rhizospheric regions in a large population and interact with the host plant in a number of ways, depending on the multitude of signals and signal perception between both partners (Petrini 1986; Jain et al. 2016). In a recent review conducted by Jia et al. (2016), the relationship of endophytic fungi and medicinal plant has been very well explained.

The symbiotic interaction of plant and fungi provides a promising strategy for the better establishment of tissue culture raised plants, enhancing various phytochemicals and reducing yield losses in cultivated crops grown under saline

environment (Varma et al. 2012b; Bajaj et al. 2014; Sharma et al. 2014a, b, 2015; Rodriguez et al. 2004). The use of plants in the remediation of saline and sodic soils is an emerging low cost approach in the reclamation of abandoned irrigated fields (Kumar and Abrol 1984; Qadir et al. 2002; Tokhtarov 2004). The creation of highly productive cultivation systems through the establishment of palatable plants has been shown to remediate saline/sodic soils as well as provide an income to resource poor farmers (Dagar et al. 2004). In this respect, the importance of arbuscular mycorrhizal (AM) fungi in restoration of denuded habitats has been recognized (St. John 1998). AM fungi support plant and bacterial performance and improve soil structure (Heinonsalo et al. 2000; Meharg and Cairney 2000).

14.4 Mycorrhizal Symbiosis and Alleviation of Salt Stress

Mycorrhizas are the symbiotic association between plants and fungi that colonize the root cortical cells during periods of active plant growth. Approximately, 80% terrestrial plants and 92% terrestrial plant families have this association in their natural habitats, surveyed by Wang and Qiu (2006). These associations are thought to exist since at least 400 million years (Rodriguez and Redman 2008; Harley 1989). The symbiotic association is characterized by translocation of sugars and other carbon compounds from the plant to fungus and, in turn, the fungus facilitates the plant with acquisition of mineral nutrients from the soil, thereby providing a critical linkage between the plant, root, and the soil (Brundrett 2004). The mycorrhization improves the nutrient and water uptake, enhance resistance to soil borne diseases, and impart tolerance against extreme environments (Smith and Read 1997). The mutualism once established changes the physiology and/or morphology of roots and plants significantly, altering the root exudation (Linderman 1988; Bansal and Mukerji 1994). More than 6000 fungal species are capable of establishing mycorrhizal relationship with approximately 2,40,000 plant species (Sharma 2001). The synergism provides enormous benefits to the plant, such as absorption of toxic elements from soil, soil restoration, establishment of green cover, disease resistance, drought tolerance, etc. These microorganisms enrich the soil and provide nutrition to the plant for better growth and development, by sequestering the nutrients from soil and translocating to the plant, in turn acquiring carbon input from the plant. This reduces the dependence on chemical fertilizers. Thus, mycorrhization is a mutually beneficial process where both the partners get benefit from one another. These associations are extremely important for the countries, where large chunks of land is degraded and is not fit for cultivation, such as South Asian Association for Regional Cooperation (SAARC) countries like Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka.

14.4.1 *Arbuscular Mycorrhizal (AM) Fungi*

The AM fungi are ubiquitous soil microbes that form an integral component of terrestrial ecosystem. They form symbiotic association with plant roots of over 90% terrestrial plant species, including Angiosperms, Gymnosperms, Pteridophytes, Lycopods, and Mosses (Smith and Read 1997). AM fungi got its name from the distinct fungal tree-shaped structure that develops in plant root cells (arbus + tree). The Arbuscules are relatively short lived (less than 15 days). AM fungi are found under all climates and in all ecosystems, regardless of the type of soil, vegetation, or growing conditions. AM fungi, which are microscopic soil fungi, colonize the roots and rhizosphere and the hyphae being thinner, branch more frequently than plant roots and spread out over several centimeters in the form of ramified filaments. This extended network increases the absorptive capacity of roots and allows the plant to have better access to a greater quantity of water and minerals required for nutrition. The association thus increases water uptake and availability of nutrients to the plant, especially insoluble soil phosphate (Clark and Zeto 2000), which in turn benefits the fungus by supply of carbohydrates derived from photosynthesis (Harrison 1999). The importance of this synergism lies in enhanced nutrient availability to the plant, resulting in better growth and improved vigor of plant and translocation of carbon to the fungus in the form of sugars, amino acids, and vitamins essential for its growth (Harley and Smith 1983). Armada et al. (2015) investigated the effectiveness of drought-adapted autochthonous microorganisms [*Bacillus thuringiensis* (Bt) and a consortium of arbuscular mycorrhizal (AM) fungi] from a degraded Mediterranean area to improve plant growth and physiology in *Zea mays* under drought stress. They found that autochthonous microorganisms were useful to protect not only native plants against drought but also an agronomically important plant such as maize. Mycorrhization significantly alters the morphology and physiology of roots and plants leading to altered root exudation.

14.4.2 *Piriformospora indica*

The present chapter focuses on use of a novel AM-like-Fungus *Piriformospora indica* for phytoremediation by early intervention with selected medicinal plants. *Piriformospora indica*, an endophytic fungus of the Sebacinaceae family, was isolated from the rhizosphere soils of the woody shrubs *Prosopis juliflora* and *Zizyphus nummularia* in the sandy desert soils of Rajasthan, India (Weiß et al. 2004; Verma et al. 1998). The fungus is easily cultivable and colonizes the roots of a wide variety of plant species through directly manipulating plant hormone-signaling pathway during the course of mutualism (Varma et al. 1999). *P. indica* is able to associate itself with roots of various plant species in a manner similar to AM fungi (Varma et al. 1999, 2001; Sharma et al. 2014a; Das et al. 2012a, b; Singh et al. 2003; Franken 2012). *P. indica* promotes nutrient uptake and enhances the

growth and biomass of the plants, including monocots and dicots (Yadav et al. 2010 and Varma et al. 2000; Trivedi et al. 2016), induces early flowering (Das et al. 2012b), increases the resistance against fungal pathogens, and allows the plant to survive under stressed environment (Das et al. 2012a; Harman 2011). Ansari et al. (2013) highlighted the ethylene- and cyclophilin A (CypA)-mediated response of *P. indica* for sustainable crop production under adverse environmental conditions. The fungus grows inter- and intracellularly and forms pear-shaped, auto-fluorescent chlamydospores within the cortex of the colonized roots and in the rhizosphere zone, but it does not invade the endodermis and the aerial parts of the plants (Varma et al. 2012a, b; Siddhanta et al. 2017). Hence, *P. indica* is a novel mutualistic symbiont in contrast to known mycorrhizas, and root nodulating bacteria. *P. indica* contains a high affinity Pi transporter (PiPT) involved in improving Pi nutrition levels in the host plant under phosphorus limiting conditions (Johri et al. 2015). This fungus provides a promising model organism for the investigations of beneficial plant microbe interaction and enables the identification of compounds which improve plant growth and productivity.

The prospects for improved agriculture, by the use of microbial inoculants as biofertilizers or biological control agents, are particularly good in less intensive, low-input agricultural systems (Bhardwaj et al. 2014). Hence, in developing countries, microbial inoculation of plants could be of great importance. The production of microbial inoculants is not very difficult; unsophisticated fermentors of modest volume can be used to produce significant quantities of bioinoculants. Present study was undertaken to investigate the effect of consortium of *P. indica* and *Azotobacter chroococcum* on salinity stress tolerance of important medicinal plants. Both inoculated and non-inoculated plantlets were subjected to four levels of salinity treatment—0, 100, 200, and 300 mM NaCl. This was achieved by early interaction of the selected medicinal plants, namely, *Glycyrrhiza glabra*, *Aloe vera*, *Bacopa monnieri*, *Asparagus racemosus*, *Coleus forskohlii*, *Withania somnifera*, *Vinca rosea*, and *Ocimum* sp. with *P. indica* and *A. chroococcum* under in vitro (plant tissue culture), in vivo (greenhouse), and field conditions in Amity University, Noida, UP, India and Issapur, New Delhi, respectively. For development of different formulations, *P. indica* was grown in large-scale fermentor. The formulated inoculum produced along with the culture filtrate was used for early intervention during the growth of plants. The plants were intervened both under in vitro (tissue culture) and ex vitro (greenhouse) conditions. The treated plants were then transferred to field for further evaluation at Issapur. Plant growth was assessed on the basis of plant biomass and other morphological parameters. Quantity of formulation required for treatment has been standardized for large number of plants. The plants were also transplanted onsite at Issapur in saline field. The plants treated with *P. indica* (primed with *Azotobacter*) performed better as compared to control (Tables 14.1, 14.2, 14.3, 14.4, 14.5, 14.6, 14.7).

Table 14.1 Growth parameters for tissue culture raised plants grown on 100 ppm salt stress in modified medium

S. No.	Plant		Shoot length (cm)	Root length (cm)	No. of leaves	Fresh wt (gm)	Dry wt (gm)	Moisture content (%)
1	<i>Coleus forskohlii</i>	Treated	2.1	0.8	6.1	0.95	0.12	87.36
		Control	1.6	0.5	4.3	0.82	0.09	73.00
2	<i>Bacopa monnieri</i>	Treated	3.6	0.6	15.55	0.98	0.15	82.69
		Control	2.2	0.4	9.6	0.89	0.11	76.40
3	<i>Ocimum sanctum</i>	Treated	1.6	0.4	6.1	0.65	0.10	84.61
		Control	1.2	0.15	4.2	0.58	0.12	79.31
4	<i>Aloe vera</i>	Treated	3.7	1.05	6.1	1.25	0.10	92.00
		Control	3.2	0.91	4.3	1.11	0.20	81.98
5	<i>Withania somnifera</i>	Treated	1.45	0.44	6.2	0.56	0.06	89.28
		Control	1.25	0.32	4.3	0.49	0.11	77.55
6	<i>Catharanthus roseus</i>	Treated	1.85	0.32	6.3	0.58	0.09	84.48
		Control	1.65	0.26	4.11	0.49	0.12	75.51

The treated plants were treated with *P. indica* primed with *Azotobacter*. Data was recorded after 2 weeks of salt treatment. Individual datum is replicate of 10 plants, $n = 3$

Table 14.2 Growth parameters for tissue culture raised plants grown on 200 ppm salt stress in modified medium

S. No.	Plant		Shoot length (cm)	Root length (cm)	No. of leaves	Fresh wt (gm)	Dry wt (gm)	Moisture content (%)
1	<i>Coleus forskohlii</i>	Treated	1.8	0.6	4.2	0.87	0.15	82.75
		Control	1.2	0.3	2.01	0.78	0.18	76.92
2	<i>Bacopa monnieri</i>	Treated	3.1	0.41	8.1	0.89	0.16	82.02
		Control	1.6	0.26	4.7	0.80	0.20	75.00
3	<i>Ocimum sanctum</i>	Treated	1.12	0.23	4.12	0.61	0.10	83.60
		Control	0.97	0.09	3.09	0.49	0.11	77.55
4	<i>Aloe vera</i>	Treated	3.11	0.85	4.2	0.98	0.18	81.63
		Control	2.78	0.66	3.12	0.78	0.18	76.92
5	<i>Withania somnifera</i>	Treated	1.15	0.23	3.67	0.51	0.10	80.39
		Control	0.95	0.16	2.42	0.44	0.11	75.00
6	<i>Catharanthus roseus</i>	Treated	1.16	0.28	5.51	0.51	0.10	80.39
		Control	1.01	0.21	3.54	0.44	0.12	72.72

The treated plants were treated with *P. indica* primed with *Azotobacter*. Data was recorded after 2 weeks of salt treatment. Individual datum is replicate of 10 plants, $n = 3$

Table 14.3 Growth parameters for tissue culture raised plants grown on 300 ppm salt stress in modified medium

S. No.	Plant		Shoot length (cm)	Root length (cm)	No. of leaves	Fresh wt (gm)	Dry wt (gm)	Moisture content (%)
1	<i>Coleus forskohlii</i>	Treated	1.44	0.32	3.12	0.69	0.12	82.60
		Control	0.61	0.11	1.01	0.49	0.12	75.51
2	<i>Bacopa monnieri</i>	Treated	2.14	0.21	3.23	0.61	0.12	80.32
		Control	0.89	0.06	0.54	0.44	0.14	68.18
3	<i>Ocimum sanctum</i>	Treated	0.62	0.13	1.12	0.52	0.10	80.76
		Control	0.47	0.03	0.59	0.48	0.15	68.75
4	<i>Aloe vera</i>	Treated	2.16	0.65	2.12	0.78	0.13	83.33
		Control	1.18	0.46	1.16	0.66	0.15	77.27
5	<i>Withania somnifera</i>	Treated	0.92	0.19	0.97	0.42	0.08	80.95
		Control	0.89	0.12	0.52	0.32	0.09	74.19
6	<i>Catharanthus roseus</i>	Treated	1.04	0.21	1.11	0.46	0.08	82.60
		Control	0.98	0.17	1.01	0.41	0.10	75.60

The treated plants were treated with *P. indica* primed with *Azotobacter*. Data was recorded after 2 weeks of salt treatment. Individual datum is replicate of 10 plants. $n = 3$

Table 14.4 Growth parameters for cutting raised plants grown on 100 ppm salt stress

S. No.	Plant		Shoot length (cm)	Root length (cm)	No. of leaves	Fresh wt (gm)	Dry wt (gm)	Moisture content (%)
1	<i>Coleus forskohlii</i>	Treated	15.81	7.67	21.66	28.91	4.85	83.22
		Control	13.65	6.32	18.88	26.21	4.99	80.96
2	<i>Bacopa monnieri</i>	Treated	12.08	3.22	45.11	14.85	2.67	82.02
		Control	11.02	2.45	39.61	13.12	2.77	78.88
3	<i>Ocimum sanctum</i>	Treated	10.12	5.67	22.81	19.26	4.20	78.19
		Control	8.98	4.98	19.12	18.43	4.98	72.97
4	<i>Aloe vera</i>	Treated	16.96	6.61	7.81	33.89	6.10	82.00
		Control	14.12	5.87	6.78	31.26	6.91	77.89
5	<i>Withania somnifera</i>	Treated	12.12	7.88	41.22	14.23	2.59	81.79
		Control	11.19	6.99	38.54	12.98	2.97	77.11
6	<i>Catharanthus roseus</i>	Treated	12.11	6.82	36.61	16.66	2.98	82.11
		Control	11.18	6.23	33.86	14.94	3.01	79.85
7	<i>Glycyrrhiza glabra</i>	Treated	9.35	8.22	14.61	12.65	2.63	79.20
		Control	8.78	7.98	12.32	11.18	2.69	75.93
8	<i>Asparagus racemosus</i>	Treated	18.21	10.11	96.12	16.16	2.89	82.11
		Control	16.56	9.87	85.26	14.85	2.87	80.67

The treated plants were treated with *P. indica* primed with *Azotobacter*. Data was recorded after 4 weeks of salt treatment. Individual datum is replicate of 6 plants, $n = 3$

Table 14.5 Growth parameters for cutting raised plants grown on 200 ppm salt stress

S. No.	Plant		Shoot length (cm)	Root length (cm)	No. of leaves	Fresh wt (gm)	Dry wt (gm)	Moisture content (%)
1	<i>Coleus forskohlii</i>	Treated	12.65	6.12	14.91	19.67	3.81	80.63
		Control	10.11	5.11	9.81	17.41	3.92	77.48
2	<i>Bacopa monnieri</i>	Treated	10.11	3.02	21.15	10.63	2.57	75.82
		Control	9.02	2.17	19.52	9.18	2.68	70.80
3	<i>Ocimum sanctum</i>	Treated	8.63	4.17	12.31	14.26	3.02	78.82
		Control	7.21	3.18	10.11	13.13	3.98	69.68
4	<i>Aloe vera</i>	Treated	14.12	5.16	5.62	26.49	5.80	78.10
		Control	12.73	4.65	4.14	23.11	5.81	74.85
5	<i>Withania somnifera</i>	Treated	10.02	5.34	21.44	11.15	2.89	74.08
		Control	9.01	3.88	19.02	10.21	2.98	70.81
6	<i>Catharanthus roseus</i>	Treated	9.12	4.12	24.21	12.55	2.76	78.00
		Control	8.11	3.06	20.23	11.12	2.91	73.83
7	<i>Glycyrrhiza glabra</i>	Treated	7.30	6.09	12.51	10.31	2.46	76.13
		Control	6.12	5.87	10.13	9.12	2.56	71.92
8	<i>Asparagus racemosus</i>	Treated	14.11	9.01	63.34	13.28	2.89	78.23
		Control	12.21	7.78	55.78	11.45	2.87	74.93

The treated plants were treated with *P. indica* primed with *Azotobacter*. Data was recorded after 4 weeks of salt treatment. Individual datum is replicate of 6 plants, $n = 3$

Table 14.6 Growth parameters for cutting raised plants grown on 300 ppm salt stress

S. No.	Plant		Shoot length (cm)	Root length (cm)	No. of leaves	Fresh wt (gm)	Dry wt (gm)	Moisture content (%)
1	<i>Coleus forskohlii</i>	Treated	9.01	4.02	5.31	10.18	3.88	61.88
		Control	6.97	3.41	3.21	7.32	3.17	56.69
2	<i>Bacopa monnieri</i>	Treated	2.21	1.01	NA	NA	NA	NA
		Control	1.23	0.97	NA	NA	NA	NA
3	<i>Ocimum sanctum</i>	Treated	4.12	2.11	NA	NA	NA	NA
		Control	3.01	1.38	NA	NA	NA	NA
4	<i>Aloe vera</i>	Treated	7.34	4.19	3.22	16.19	4.12	74.55
		Control	6.67	3.46	2.44	14.04	4.91	65.02
5	<i>Withania somnifera</i>	Treated	7.21	4.32	12.43	8.09	2.28	71.81
		Control	5.91	2.67	9.01	6.91	2.45	64.54
6	<i>Catharanthus roseus</i>	Treated	8.11	3.82	8.13	9.66	2.26	76.60
		Control	7.41	3.06	6.21	8.34	2.43	70.86
7	<i>Glycyrrhiza glabra</i>	Treated	4.31	1.01	NA	NA	NA	NA
		Control	3.92	0.88	NA	NA	NA	NA
8	<i>Asparagus racemosus</i>	Treated	9.12	6.01	21.15	9.09	2.54	72.05
		Control	7.09	3.18	12.09	7.15	2.47	65.45

The treated plants were treated with *P. indica* primed with *Azotobacter*. Data was recorded after 4 weeks of salt treatment. Individual datum is replicate of 6 plants, $n = 3$

Table 14.7 Growth parameters for cutting raised plants grown on saline soil at Issapur

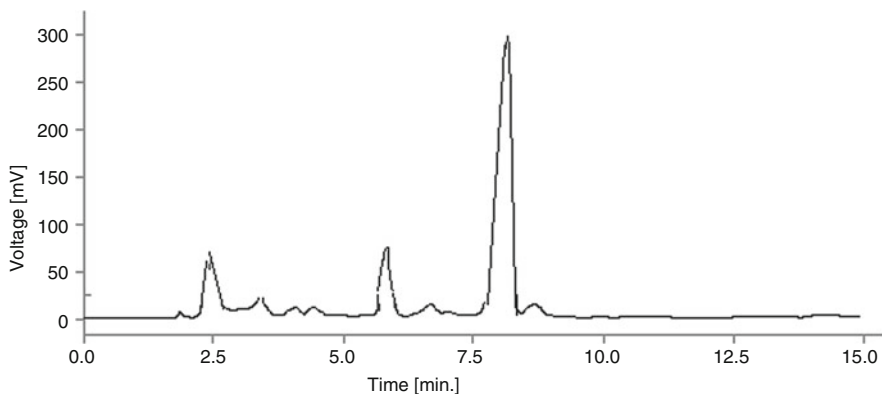
S. No.	Plant		Shoot length (cm)	Root length (cm)	No. of leaves	No. of flowers	Fresh wt (gm)	Dry wt (gm)	Moisture content (%)	% Survival
1	<i>Coleus forskohlii</i>	Treated	9.01	4.02	5.31	0	10.18	2.88	71.65	21.6
		Control	6.97	3.41	3.21	0	7.32	2.84	61.20	7.2
2	<i>Bacopa monnieri</i>	Treated	NA	NA	NA	0	NA	NA	NA	0
		Control	NA	NA	NA	0	NA	NA	NA	0
3	<i>Ocimum sanctum</i>	Treated	4.12	2.11	8.23	0	7.45	2.42	69.37	2.8
		Control	3.01	1.38	7.01	0	6.24	2.16	65.38	27.14
4	<i>Aloe vera</i>	Treated	7.34	4.19	3.22	0	16.19	3.12	80.72	56
		Control	6.67	3.46	2.44	0	14.04	4.01	71.43	38.6
5	<i>Withania somnifera</i>	Treated	7.21	4.32	12.43	0	8.09	2.28	71.81	29.2
		Control	5.91	2.67	9.01	0	6.91	2.45	64.54	18.5
6	<i>Catharanthus roseus</i>	Treated	8.11	3.82	8.13	8.22	9.66	2.26	76.60	21.66
		Control	7.41	3.06	6.21	3.11	8.34	2.43	70.86	6.12
7	<i>Glycyrrhiza glabra</i>	Treated	NA	NA	NA	NA	NA	NA	NA	0
		Control	NA	NA	NA	NA	NA	NA	NA	0
8	<i>Asparagus racemosus</i>	Treated	9.12	6.01	21.15	0	9.09	2.54	72.45	56
		Control	7.09	3.18	12.09	0	7.15	2.47	65.45	10

The treated plants were treated with *P. indica* primed with *Azotobacter*. Data was recorded after 26 weeks onwards of salt treatment. Individual datum is replicate of 6 plants, $n = 3$

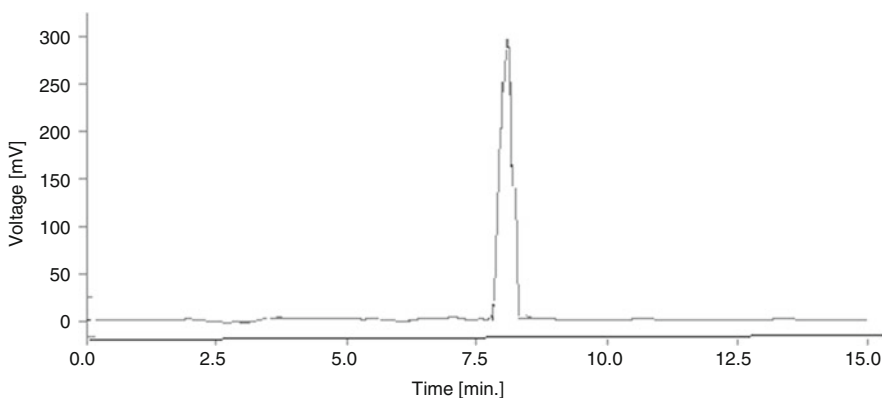
14.5 Chemical Analysis of Secondary Metabolites

14.5.1 *Coleus forskohlii*: Root Harvested from Saline Soil After 6 Months Were Subjected to Forskoline Analysis

Standard



Treated



Details: *Coleus forskohlii* roots in HPLC for Forskoline

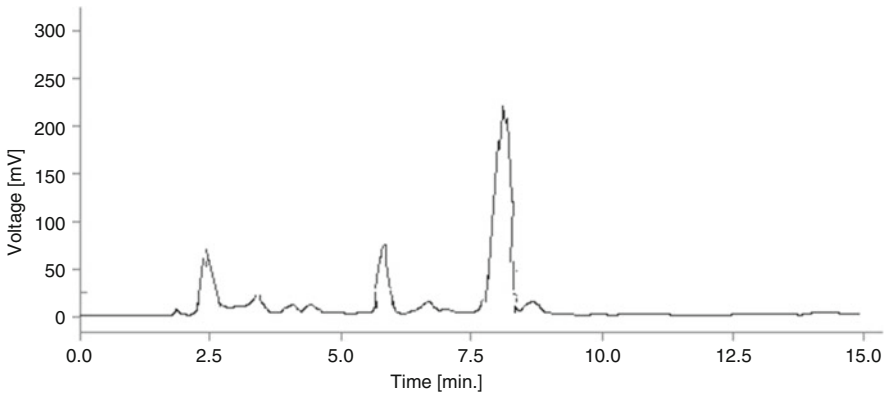
Conc. of sample 1.56512 gm/100 ml

Loss on drying = 40.1%

Forskoline content on L.O.D. basis: 0.85%

Forskoline content on fresh weight basis 8.19%

Control

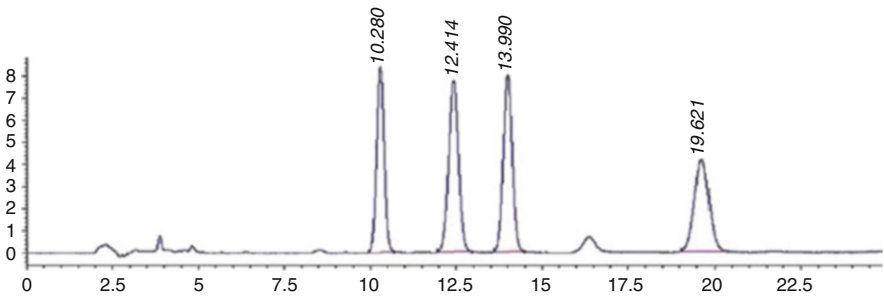


Coleus forskohlii Roots in HPLC for Forskoline Details: Conc. of sample 1.56891 gm/100 ml Loss on drying = 4.27% Forskoline content on L.O.D. basis: 0.68% Forskoline content on fresh weight basis 6.53%

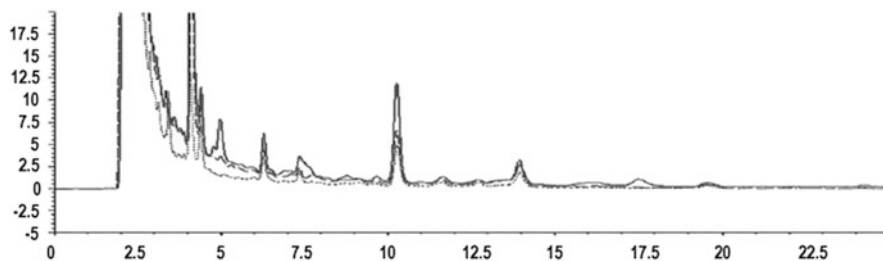
The treated plants produced 25.47% more forskoline as compared to untreated control under saline conditions.

14.5.2 *Vinca rosea*: Leaves were Harvested from Plants Growing in Saline Soil After 6 Months and Subjected to Analysis of Vindoline, Vincristine, Catharanthine, and Vinblastine

Standard



Treated



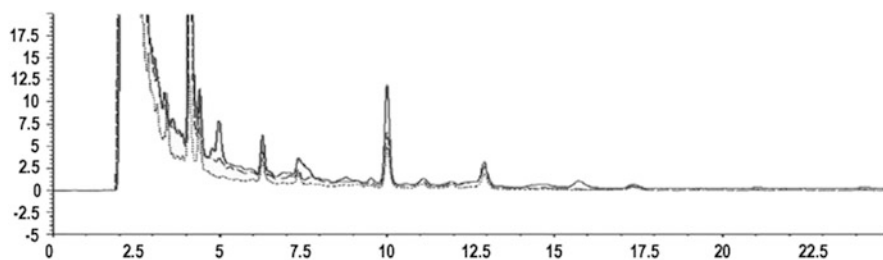
Vinca rosea leaves in HPLC for Vindoline, Vincristine, Catharanthine, and Vinblastine Details: Conc. of sample 0.5871 gm/100 ml
 Loss on drying = 3.72%
 Alkaloid content on L.O.D. basis:

Vindoline	Vincristine	Catharanthine	Vinblastine
0.056	0.0013	0.016	0.0017

Alkaloid content on fresh weight basis:

Vindoline	Vincristine	Catharanthine	Vinblastine
0.51%	0.013%	0.15%	0.018%

Control



Vinca rosea leaves in HPLC for Vindoline, Vincristine, Catharanthine, and Vinblastine
 Details: Conc. of sample 0.5831 gm/100 ml
 Loss on drying = 3.69%
 Alkaloid content on L.O.D. basis:

Vindoline	Vincristine	Catharanthine	Vinblastine
0.033	0.0007	0.0087	0.0012

Alkaloid content on fresh weight basis:

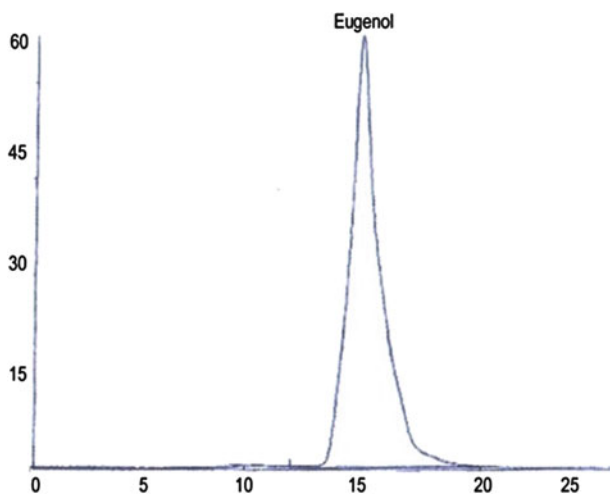
Vindoline	Vincristine	Catharanthine	Vinblastine
0.32%	0.008%	0.09%	0.011%

Alkaloid content treated vs control:

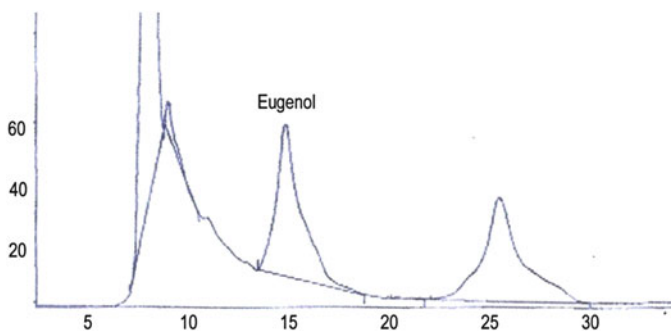
S.No	Alkaloid	% Change
1	Vindoline	59%
2	Vincristine	62.5%
3	Catharanthine	66.6%
4	Vinblastine	63.6%

14.5.3 Ocimum sanctum: Leaves Were Harvested from Plants Growing in Saline Soil After 6 Months and Subjected to Analysis of Eugenol

Standard



Treated



Ocimum sanctum leaves in HPLC for Eugenol analysis

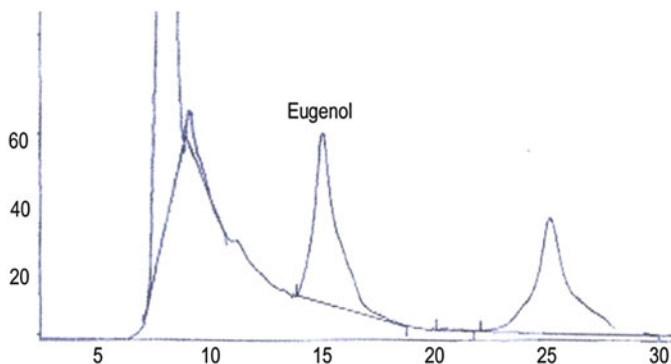
Details: Conc. of sample 1.2865 gm/100 ml

Loss on drying = 3.93%

Eugenol content on L.O.D. basis: 0.27%

Eugenol content on fresh weight basis: 2.53%

Control



Ocimum sanctum leaves in HPLC for Eugenol analysis

Details: Conc. of sample 1.2845 gm/100 ml

Loss on drying = 3.87%

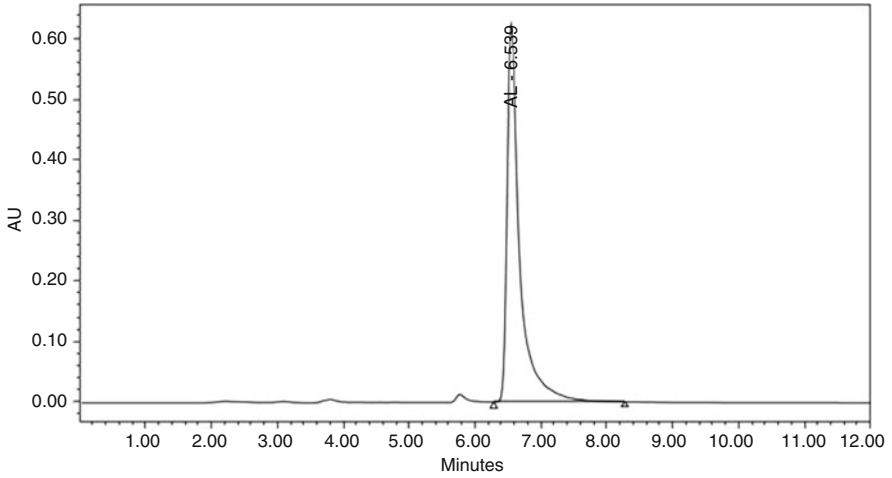
Eugenol content on L.O.D. basis: 0.21%

Eugenol content on fresh weight basis: 2.18%

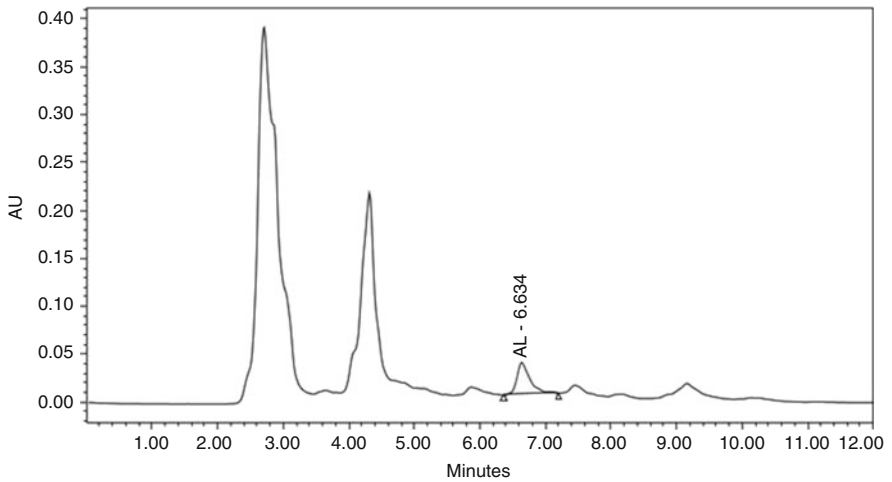
The treated plants produced 28.57% more Eugenol as compared to untreated control under saline conditions.

14.5.4 Aloe vera: Leaves Were Harvested from Plants Growing in Saline Soil After 12 Months and Subjected to Analysis of Aloin

Standard



Treated



Aloe vera leaves in HPLC for Aloin analysis

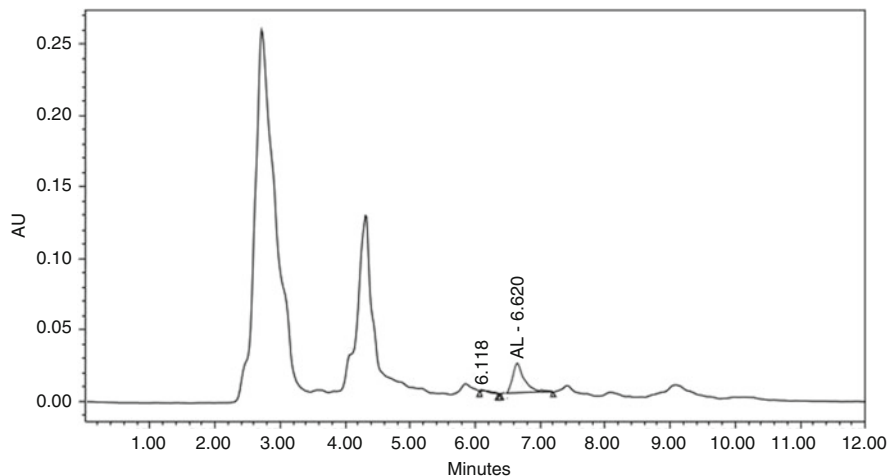
Details: Conc. of sample 0.4365 gm/100 ml

Loss on drying = 2.05%

Aloin content on L.O.D. basis: 0.11%

Aloin content on fresh weight basis: 1.03%

Control



Aloe vera leaves in HPLC for Aloin analysis

Details: Conc. of sample 0.4123 gm/100 ml

Loss on drying = 2.01%

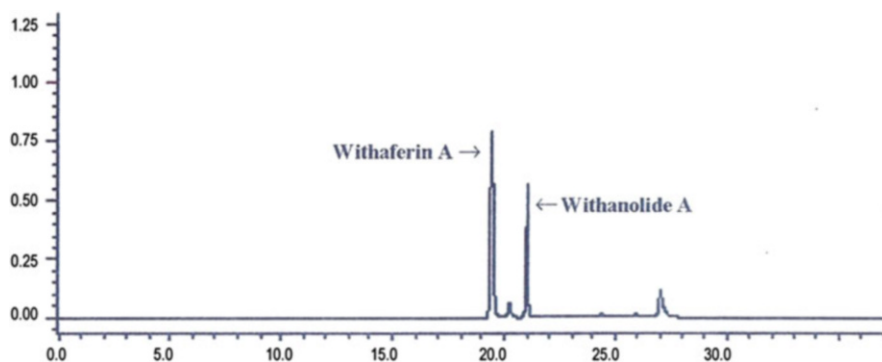
Aloin content on L.O.D. basis: 0.06%

Aloin content on fresh weight basis: 0.66%

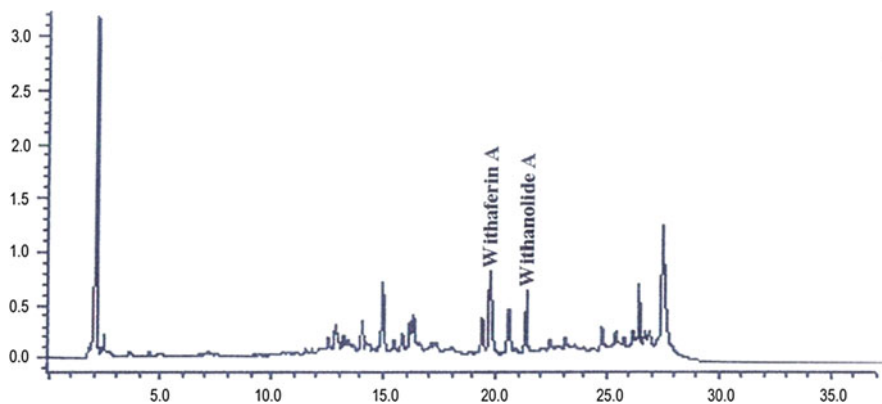
The treated plants produced 56.06% more Aloin as compared to untreated control under saline conditions.

14.5.5 *Withania somnifera*: Roots Were Harvested from Plants Growing in Saline Soil After 4 Months and Subjected to Analysis of Withaferin A and Withanolide A

Standard



Treated



Withania somnifera roots in HPLC for Withaferin A and Withanolide A analysis

Details: Conc. of sample 1.4213 gm/100 ml

Loss on drying = 3.51%

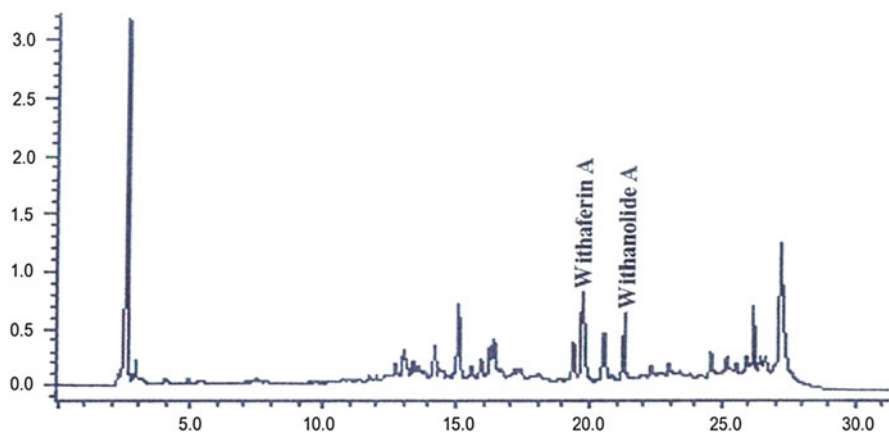
Alkaloid content on L.O.D. basis:

S.No	Alkaloid	Content
1	Withaferin A	0.073
2	Withanolide A	0.055

Alkaloid content on fresh weight basis:

S.No	Alkaloid	Content
1	Withaferin A	0.72
2	Withanolide A	0.56

Control



Withania somnifera roots in HPLC for Withaferin A and Withanolide A analysis

Details: Conc. of sample 1.3917 gm/100 ml

Loss on drying = 3.45%

Alkaloid content on L.O.D. basis:

S.No	Alkaloid	Content
1	Withaferin A	0.055
2	Withanolide A	0.041

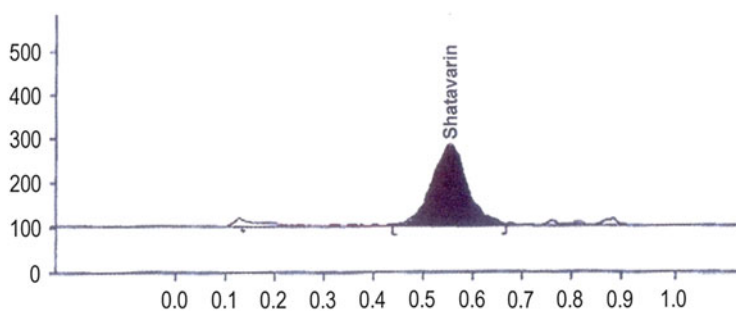
Alkaloid content on fresh weight basis:

S.No	Alkaloid	Content
1	Withaferin A	0.54
2	Withanolide A	0.41

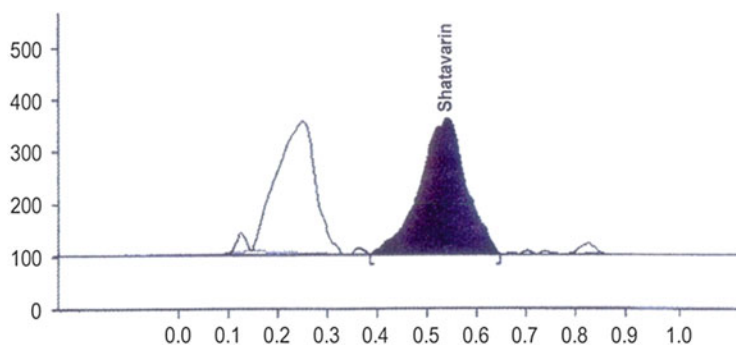
The treated plants produced 33.3% more Withaferin A and 36.5 % more Withanolide A as compared to untreated control under saline conditions.

14.5.6 *Asparagus racemosus*: Roots Were Harvested from Plants Growing in Saline Soil After 18 Months and Subjected to Analysis of Shatavarin

Standard



Treated



Asparagus racemosus roots in HPLC for Shatavarin analysis

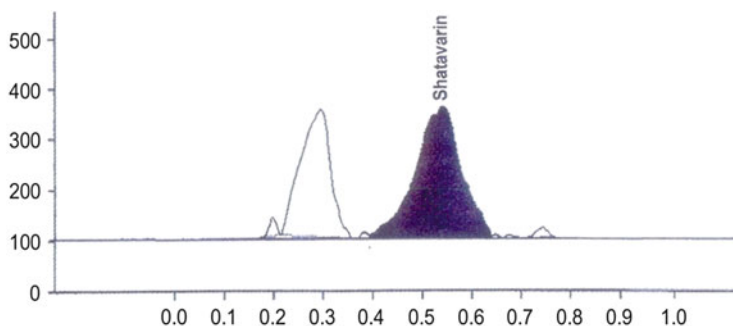
Details: Conc. of sample 1.631 gm/100 ml

Loss on drying = 3.74%

Shatavarin content on L.O.D. basis: 0.41%

Shatavarin content on fresh weight basis: 4.21%

Control



Asparagus racemosus roots in HPLC for Shatavarin analysis

Details: Conc. of sample 1.541 gm/100 ml

Loss on drying = 3.68%

Shatavarin content on L.O.D. basis: 0.32%

Shatavarin content on fresh weight basis: 3.19%

The treated plants produced 31.97% more Shatavarin as compared to untreated control under saline conditions.

14.6 Discussion and Analysis

On interaction of *P. indica* with different strains of Plant Growth Promoting Rhizobacteria (PGPRs), it was observed that the growth was completely blocked by *Pseudomonas fluorescense*; however, *Azotobacter chroococcum* promoted the growth of the fungus (Pham et al. 2004). In the present study, *A. chroococcum* primed *P. indica* has considerable improvement in plant biomass and productivity as compared to untreated control both under simulated saline conditions and in field. *P. indica* has been reported to induce resistance in the monocotyledonous plant barley to fungal diseases, along with tolerance to salt stress without affecting the plant productivity (Waller et al. 2005; Tuteja 2007).

Soil salinity is one of the major constraints that affect the growth, yield, and survival of plants. The lower water potential in the roots under salinity stress causes stomatal closure and suppression of mesophyll conductance. This negatively affects the photosynthetic rate and limits CO₂ assimilation (Ashraf and Harris 2013; Fletcher et al. 2007). The immediate effect of salt stress is a lower biomass, stunted growth, and reduced chlorophyll content. Low and moderate salinity stress (100 and 200 mM NaCl) caused a low, but not significant reduction in growth parameters and biomass. High salinity stress (300 mM) in contrast caused considerable disturbance

of the physiology of both inoculated and non-inoculated plantlets. The enhancement in growth parameters of inoculated plantlets may partially be attributed to obligatory endomycotic bacteria, associated with *P. indica* (Sharma et al. 2008).

The significant increase in length of roots of consortium inoculated plantlets can be attributed to production of auxin by *P. indica* (Sirrenberg et al. 2007; Dong et al. 2013). Waller et al. (2005) found that *P. indica* inoculated barley plantlets could tolerate moderate salinity stress (100 mM) in hydroponic culture. In the present study, it was found that *P. indica* was able to protect plantlets to some degree even at high salinity (300 mM). An earlier study showed that *A. vera* plantlets inoculated with *P. indica* had appreciably higher gel and aloin (anthraquinone derivative) content (Sharma et al. 2014a, 2015). *Aloe vera* plantlets inoculated with *P. indica* had significantly higher secondary metabolite content and antioxidant activity (Sharma et al. 2016).

There are only a few reports on the potential application of *P. indica* for enhancing secondary metabolite content of plants (Satheesan et al. 2012; Bajaj et al. 2014; Sharma et al. 2014a, 2015; Kilam et al. 2015; Gill et al. 2016) under salinity stress. Thus, efforts have been made with the consortium of *P. indica* and *Azotobacter* with various plants having great medicinal importance, under saline stress.

In the present study, the increase in secondary metabolites could be due to elicitation of plant defense in response to fungal elicitors like lipopolysaccharides and glycoproteins formed by the action of plant-derived hydrolases secreted in response to endophyte colonization (Gao et al. 2010). The inoculated plantlets also had significantly higher radical scavenging activity in terms of greater inhibition of free radicals, which could be due to greater secondary metabolite content, i.e., phenolics, flavonoids, and flavonols. This imparts greater ability to reduce oxidative damage associated with many phyto-pathogenic diseases (Teshome et al. 2015; Nath et al. 2016)). The results presented in this study confirm that NaCl stress disrupts nutrient and water acquisition, resulting in reduced growth and biomass of plantlets. However, plant tolerance to salinity stress is improved by its association with symbiotic fungus *P. indica*. The interaction of Plantlets with the consortium resulted in improved morphological features, enhanced biomass, greater host defense, more survival, and increased photosynthetic activity. This interaction has been found successful in terms of protecting the plant not only at moderate but even at higher concentrations of NaCl. The mechanisms of stress alleviation by the consortium could be due to improved nutrient uptake leading to growth promotion and enhanced radical scavenging capacity and secondary metabolites that help the plant resist the damaging effects of salt stress. The potential of consortium of *P. indica* and *Azotobacter* in reducing the problems caused by salinity stress and protecting the crops in arid and semi-arid agricultural regions is worthy of more detailed research.

References

- Aggarwal A, Kadian N, Karishma N, Tanwar A, Gupta KK (2012) Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. *J Appl Nat Sci* 4:144–155
- Ansari MW, Trivedi DK, Sahoo RK, Gill SS, Tuteja NA (2013) Critical review on fungi mediated plant responses with special emphasis to *Piriformospora indica* on improved production and protection of crops. *Plant Physiol Biochem* 70:403–410
- Armada E, Azcón R, López-Castillo OM, Calvo-Polanco M, Ruiz-Lozano JM (2015) Autochthonous arbuscular mycorrhizal fungi and *Bacillus thuringiensis* from a degraded Mediterranean area can be used to improve physiological traits and performance of a plant of agronomic interest under drought conditions. *Plant Physiol Biochem* 90:64–74
- Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Sci* 166:3–16
- Ashraf M, Harris PJC (2013) Photosynthesis under stressful environments: an overview. *Photosynthetica* 51:163–190
- Ashraf M, Tufail M (1994) Variation in salinity tolerance in sunflower (*Helianthus annuus* L.) *J Agron Soil Sci* 174:351–362
- Bajaj R, Agarwal A, Rajpal K, Asthana S, Prasad R, Kharkwal AC, Kumar R, Sherameti I, Oelmüller R, Varma A (2014) Co-cultivation of *Curcuma longa* with *Piriformosporaindica* enhances the yield and active ingredients. *Am J Curr Microbiol* 2:6–17
- Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel KH, Schäfer P, Schwarczinger I, Zuccaro A, Skoczowski A (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytol* 180:501–510
- Bansal M, Mukerji KG (1994) Efficacy of root litter as a biofertilizer. *Biol Fertil Soils* 18:228–230
- Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb Cell Fact* 13:66
- Brundrett M (2004) Diversity and classification of mycorrhizal associations. *Biol Rev* 79:473–495
- Chen X, Gu Z, Xin D, Hao L, Liu C, Huang J, Ma B, Zhang H (2011) Identification and characterization of putative CIPK genes in maize. *J Genet Genomics* 38:77–87
- Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. *J Plant Nutr* 23:867–902
- Dagar JC, Tomar OS, Kumar Y, Yadav RK (2004) Growing three aromatic grasses in different alkali soils in semi-arid regions of northern India. *Land Degrad Dev* 15:143–151
- Das A, Sheramati I, Varma A (2012a) Contaminated soils: physical, chemical and biological components. In: Varma A, Kothe E (eds) *Bio-geo interactions in metal-contaminated soils*. Springer, Heidelberg, pp 1–16
- Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri AK, Varma A (2012b) The root endophyte fungus *Piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. *Plant Signal Behav* 7:1–10
- Dong S, Tian Z, Chen PJ, Kumar RS, Shen CH, Cai D, Oelmüller R, Yeh KW (2013) The maturation zone is an important target of *Piriformospora indica* in Chinese cabbage roots. *J Exp Bot* 64:4529–4540
- Evelin H, Kapoor R (2014) Arbuscular mycorrhizal symbiosis modulates antioxidant response in salt-stressed *Trigonella foenum-graecum* plants. *Mycorrhiza* 24:197–208
- Fletcher AL, Sinclair TR, Allen LH (2007) Transpiration responses to vapour pressure deficit in well watered ‘slow-wilting’ and commercial soybean. *Environ Exp Bot* 61:145–151
- Franken P (2012) The plant strengthening root endophyte *Piriformospora indica*: potential application and the biology behind. *Appl Microbiol Biotechnol* 96:1455–1464
- Galvani A (2007) The challenge of the food sufficiency through salt tolerant crops. *Rev Environ Sci Biotechnol* 6:3–16

- Gao FK, Dai CC, Liu XZ (2010) Mechanisms of fungal endophytes in plant protection against pathogens-review. *Afr J Microbiol Res* 4:1346–1351
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930
- Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. *Front Microbiol* 7:332. doi:10.3389/fmicb.2016.00332
- Harley JL (1989) The significance of mycorrhiza. *Mycol Res* 92:129–139
- Harley, JL, Smith SE (1983) Mycorrhizal symbiosis, 1st edn. Academic Press, London
- Harman GE (2011) Multifunctional fungal plant symbiont: new tools to enhance plant growth and productivity. *New Phytol* 189:647–649
- Harrison MJ (1999) Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annu Rev Plant Physiol Plant Mol Biol* 50:361–389
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annu Rev Plant Physiol Plant Mol Biol* 51:463–499
- Heinonsalo J, Jorgensen KS, Hahtela K, Sen R (2000) Effects of *Pinu sylvestris* root growth and mycorrhizosphere development on bacterial carbon source utilization and hydrocarbon oxidation in forest and petroleum-contaminated soils. *Can J Microbiol* 46:451–464
- Hu Y, Schmidhalter U (1997) Interactive effects of salinity and macronutrient level on wheat. II. Composition. *J Plant Nutr* 20:1169–1182
- Jain S, Varma A, Tuteja N, Choudhary DK (2016) Plant growth promoting microbial-mediated induced systemic resistance in plants: induction, mechanism and expression. In: Choudhary DK, Varma A (eds) *Microbial-mediated induced systemic resistance in plants*. Springer, Singapore, pp 213–226
- Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Front Microbiol* 7:906. doi:10.3389/fmicb.2016.00906
- Jin ZM, Wang CH, Liu ZP, Gong WJ (2007) Physiological and ecological characters studies on *Aloe vera* under soil salinity and sea water irrigation. *Process Biochem* 42:710–714
- Johri AK, Oelmüller R, Dua M, Yadav V, Kumar M, Tuteja N, Varma A, Bonfante P, Persson BL, Stroud RM (2015) Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front Microbiol* 6:984. doi:10.3389/fmicb.2015.00984
- Kilam D, Saifi M, Abdin MZ, Agnihotri A, Varma A (2015) Combined effects of *Piriformospora indica* and *Azotobacter chroococcum* enhance plant growth, antioxidant potential and steviol glycoside content in *Stevia rebaudiana*. *Symbiosis* 66:149–156
- Kumar A, Abrol IP (1984) Studies on the reclaiming effort of Kamal grass and para-grass grown on highly sodic soil. *Indian J Agric Sci* 54:189–193
- Linderman RG (1988) Mycorrhizal interactions with rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* 78:366–371
- Lozano JMR (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza* 13:309–317
- Meharg AA, Cairney JWG (2000) Ectomycorrhizas – extending the capabilities of rhizosphere remediation? *Soil Biol Biochem* 32:1475–1484
- Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora indica* interaction under stress condition. *Front Plant Sci* 7:1574. doi:10.3389/fpls.2016.01574. (Published 18 Oct 2016)
- Petrini O (1986) Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema NJ, Van den Huevel J (eds) *Microbiology of the phyllosphere*. Cambridge University Press, Cambridge, pp 175–187
- Pham HG, Singh A, Malla R, Kumari R, Prasad R, Sachdev M, Rexer KH, Kost G, Luis P, Kaldorf M, Buscot F, Herrmann S, Peškan T, Oelmüller R, Saxena AK, Declerck S, Mittag M, Stabentheiner E, Hehl S, Varma A (2004) Interaction of *Piriformospora indica* with diverse

- microorganisms and plants. In: Varma A, Abbott LK, Werner D, Hampp R (eds) Plant surface microbiology. Springer, Berlin, pp 237–265
- Qadir M, Qureshi RH, Ahmad N (2002) Amelioration of calcareous saline-sodic soils through phytoremediation and chemical strategies. *Soil Use Manag* 18:381–385
- Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. *J Exp Bot* 59:1109–1114
- Rodriguez RJ, Redman RS, Henson J (2004) The role of fungal symbioses in the adaptation of plants to high stress environments. *Mitig Adapt Strat Glob Chang* 9:261–272
- Satheesan J, Narayanan AK, Sakunthala M (2012) Induction of root colonization by *Piriformospora indica* leads to enhanced asiaticoside production in *Centella asiatica*. *Mycorrhiza* 22:195–202
- Sharma MP (2001) Biodiversity and role of potential isolates VA-mycorrhizae in various plant species of economic value. Ph.D. Thesis, Jiwaji University, Gwalior, Centre for Mycorrhizal research, Tata Energy Research Institute, New Delhi, p 10
- Sharma M, Schmid M, Rothballer M, Hause G, Zuccaro A, Imani J, Kampfer P, Domann E, Schäfer P, Hartmann A, Kogel KH (2008) Detection and identification of bacteria intimately associated with fungi of the order Sebaciales. *Cell Microbiol* 11:2235–2246
- Sharma P, Kharkwal AC, Abdin MZ, Varma A (2014a) *Piriformospora indica* improves micro propagation, growth and phyto chemical content of *Aloe vera* L. *Plants. Symbiosis* 64:11–23
- Sharma P, Kharkwal AC, Kharkwal H, Abdin MZ, Varma A (2014b) A review on pharmacological properties of *Aloe vera*. *Int J Pharm Sci Rev Res* 29:31–37
- Sharma P, Abdin MZ, Kharkwal AC, Varma A (2015) Salt stress alleviation using mycorrhizal fungi—a short review. *Botanica* 64(65):1–8
- Sharma P, Kharkwal AC, Abdin MZ, Varma A (2016) *Piriformospora indica* mediated salinity tolerance in *Aloe vera* plantlets. *Symbiosis* 97:1–13. doi:10.1007/s13199-016-0449-0
- Shen H, Ye W, Hong L, Huang H, Wang Z, Deng X, Yang Q, Xu Z (2006) Progress in parasitic plant biology: host selection and nutrient transfer. *Plant Biol* 8:175–185
- Siddhanta S, Paidi SK, Bushley K, Prasad R, Barman I (2017) Exploring morphological and biochemical linkages in fungal growth with label-free light sheet microscopy and Raman spectroscopy. *ChemPhysChem* 18:72–78
- Singh AN, Singh AR, Kumari M, Rai MK, Varma A (2003) Biotechnology importance of *Piriformospora indica* – a novel symbiotic mycorrhiza-like fungus: an overview. *Indian J Biotechnol* 2:65–75
- Sirrenberg A, Gobel C, Grond S, Czempinski N, Ratzinger A, Karlovsky P, Santos P, Feussner I, Pawlowski K (2007) *Piriformospora indica* affects plant growth by auxin production. *Physiol Plant* 131:581–589
- Smith, SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, San Diego, p 605
- St. John T (1998) Mycorrhizal inoculation in habitat restoration. *Land Water* 42:17–19
- Sudhakar C, Lakshmi A, Giridarakumar S (2001) Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci* 161:613–619
- Teshome T, Sintayehu B, Yohannes H, Gebrelibanos M, Karim A, Gomathi P, Yarlagaadda R (2015) Radical scavenging activity and preliminary phytochemical screening on aerial part extracts of *Cineraria abyssinica* sch. bip. EXA. *J Pharmacogn Phytochem* 3:239–243
- Thrall PH, Hochberg ME, Burdon JJ, Bever JD (2007) Co evolution of symbiotic mutualists and parasites in a community context. *Trends Ecol Evol* 22:120–126
- Tokhtarov VP (2004) Sorghum: predecessor, fertilizing, soil treatment. *Sorghum and Corn*, vol 5, P1768, pp 22–24
- Trivedi DK, Srivastava A, Verma PK, Tuteja N, Gill SS (2016) *Piriformospora indica*: a friend in need is a friend in deed. *Res Rev J Bot Sci* 5:2347–2308
- Tuteja N (2007) Mechanisms of high salinity tolerance in plants. *Methods Enzymol* 428:419–438

- Varma A, Verma S, Sudha, Sahay N, Buttehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant growth promoting root endophyte. *Appl Environ Microbiol* 65:2741–2744
- Varma A, Singh A, Sudha, Sahay NS, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Hurek T, Bleichert O, Rexer KH, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2000) *Piriformospora indica*: an axenically culturable mycorrhiza-like endosymbiotic fungus (Chap 8). In: Hock B (ed) *Mycota IX*. Springer, Heidelberg, pp 225–253
- Varma A, Singh A, Sudha Sahay N, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T, Bleichert O, Rexer KH, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica*: an axenically culturable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) *The mycota IX, fungal associations*. Springer, Berlin, pp 123–150
- Varma A, Sherameti I, Tripathi S, Prasad R, Das A, Sharma M, Bakshi M, Johnson JM, Bhardwaj S, Arora M, Rastogi K, Agrawal A, Kharkwal AC, Talukdar S, Bagde US, Bisaria VS, Upadhyaya CP, Won PS, Chen Y et al (2012a) The symbiotic fungus *Piriformospora indica*: review. *Mycota* 9:231–254
- Varma A, Bakshi M, Lou B, Hartmann A, Oelmueller R (2012b) *Piriformospora indica*: a novel plant growth-promoting mycorrhizal fungus. *Agric Res* 1:117–131
- Verma S, Varma A, Rexer K, Hassel A, Kost G, Sarbhoy A, Bisen P, Buttehorn B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia* 90:896–903
- Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, Wettstein DV, Franken P, Kogel KH (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc Natl Acad Sci* 102:13386–13391
- Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16:299–363
- Weiß M, Selosse MA, Rexer KH, Urban A, Oberwinkler F (2004) Sebaciales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycol Res* 108:1002–1010
- Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *J Biol Chem* 285:26532–26544

Chapter 15

Cocultivation of *Piriformospora indica* and *Azotobacter chroococcum* for Production of Artemisinin

Prasun Bandyopadhyay, Monika Arora, M.Z. Abdin, and Ajit Varma

Abstract Artemisinin is one of the major active ingredients used in artemisinin combination therapies (ACTs) used in malarial treatment. It is produced from *Artemisia annua* L. Malaria being one of the most severe tropical diseases, dependency on the production of artemisinin has been increasing. Lower yield (0.01–1.1%) further complicates the production process. This has led to the development of alternate strategy to improve plant productivity and enhance the active ingredient. Biostimulants like *Piriformospora indica* and *Azotobacter chroococcum* have been well known for their beneficial interaction with plants. Here, we studied the impact of dual inoculation of these stimulants in the growth and productivity of artemisinin in the poly house condition. The plant growth was monitored by measuring parameters like height of plant, total dry weight, and leaf yield with an increase of 63.51, 52.61, and 79.70%, respectively, for treatment with dual biological consortium, as compared to that of control plants. This significant improvement in biomass was associated with higher total chlorophyll content (59.29%) and enhanced nutrition (especially nitrogen and phosphorus, 55.75 and 86.21%, respectively). The concentration of artemisinin along with expression patterns of artemisinin biosynthesis genes was appreciably higher in dual treatment, which showed positive correlation. The study suggested the potential use of the consortium *P. indica* strain DSM 11827 and *A. chroococcum* strain W-5 in *A. annua* L.

P. Bandyopadhyay • M. Arora • A. Varma (✉)
Amity Institute of Microbial Technology, Amity University, Sector 125, Noida, Uttar Pradesh
201303, India
e-mail: ajitvarma@amity.edu

M.Z. Abdin
Department of Biotechnology, Jamia Hamdard University, New Delhi, India

15.1 Introduction

Artemisia annua L. (sweet wormwood) is an important medicinal plant due to presence of artemisinin. It belongs to genus *Artemisia*, family Asteraceae (Compositae) with an annual growth cycle (Willcox et al. 2004). The phyto-molecule artemisinin, sesquiterpene lactone containing endoperoxide bridge, is obtained from aerial parts of *A. annua* L. plants (Mandal et al. 2015). Artemisinin is an effective anti-malarial drug discovered by Miller and Su (2011). It has been also reported that artemisinin is not only effective against malaria but also for human cancer (Singh and Lai 2004) and hepatitis B virus (Romero et al. 2005). So far artemisinin-based combination therapies (ACTs) have been the choice for the treatment of people worldwide (Abdin et al. 2003). *A. annua* L. produces small amount of artemisinin (0.01–1.1%). Such low yields of artemisinin results in relatively high cost for isolation and purification of the useful chemical. Also, the demand of artemisinin production from dried plant material of *A. annua* L. has been estimated to about 289 tons as against the annual production of about 232–262 tons (Arora et al. 2016).

Rhizosphere microbiota like arbuscular mycorrhizal fungi (AMF) are well-known plant beneficial soilborne microsymbionts. They significantly contribute toward improved agricultural performance by triggering diverse plant physiological responses. Hence, these have been employed for many agricultural production systems as well as for medicinal and endangered plant species (Pozo et al. 2010). The symbiotic association of arbuscular mycorrhizal fungi (AMF) with the plant is in synergistic coordination with the plant growth-promoting rhizobacteria (PGPR) (Bandyopadhyay et al. 2016a; Bandyopadhyay et al. 2016b; Bakker et al. 2013; Berendsen et al. 2012; Bhuyan et al. 2015). The overall plant performance relies on both bacteria and the fungi whereby the nitrogen-fixing ability of bacteria is stimulated by improved phosphate uptake due to AMF association and vice versa (Javot et al. 2007). PGPRs show phosphate-solubilizing mechanisms, enhancement in phytohormone production, increased antifungal activity, etc. (Awasthi et al. 2011; Prasad et al. 2015). The synergistic interaction between plant and microbes in rhizosphere critically improves growth and productivity of plants through an array of processes like increased nutrient uptake, availability, nitrogen fixation, nutrient recycling, photosynthetic rate, and pathogen resistance (Jeffries et al. 2003).

P. indica as well as arbuscular mycorrhiza fungi individually have also been shown to enhance artemisinin content in *A. annua* L. plants (Kapoor et al. 2007; Rapparini et al. 2008; Chaudhary et al. 2008; Sharma and Agrawal 2013). Kapoor et al. (2007) reported an increase in artemisinin concentration in leaves of *A. annua* from 0.1% (control) to 0.3% (*Glomus fasciculatum* treated) while investigating the effect of two AMF *Glomus fasciculatum* and *Glomus macrocarpum* singly and along with addition of phosphorous. The increased artemisinin concentration was attributed to high leaf yield and shoot growth which was further validated by high glandular trichome (artemisinin biosynthesis and assembly sites) density in the

mycorrhizal-treated plants. *Azotobacter* is a Gram-negative aerobic soil-dwelling nitrogen-fixing bacteria (Lakshminarayana et al. 1992). It is found in soil and water systems and in association with plants (Martyniuk and Martyniuk 2003). Only, recently studies analyzing synergistic effect of PGPRs and AMF on medicinal and crop plants have been conducted (Awasthi et al. 2011; Walker et al. 2012; Vafadar et al. 2014).

15.2 Effect of *P. indica* and *A. chroococcum* on Plant Growth Parameters

Inoculation of *A. annua* L. plants with *Piriformospora indica* and *A. chroococcum* either singly or in combination under poly house conditions improved the growth of plants in terms of plant height, biomass, and total leaf yield per plant as compared with control plants (Table 15.1). *A. annua* L. plants treated with either *P. indica* or *A. chroococcum* enhanced the growth compared with control. When combined, inoculation of plants with both *P. indica* and *A. chroococcum* was highly effective in improving the plant height, biomass, and leaf yield with an observed increase of 63.51, 52.61 and 79.70% respectively, compared with control (Table 15.1).

Rhizospheric soil from *A. annua* L. plants treated with *A. chroococcum* alone or in combination with *P. indica* was used for determination of the viable count of *A. chroococcum* by using standard serial dilution pour plate method. *A. annua* L. plants treated only with *A. chroococcum* showed 18.33×10^5 CFU/g soil, whereas dual treated plants exhibited high population of *A. chroococcum* (21.12×10^5 CFU/g soil) in the rhizospheric soil. *P. indica* colonization was evaluated by randomly selected fine roots from 2-month-old *A. annua* L. as method followed by Phillips and Hayman (1970), and percentage colonization of *P. indica* was calculated using the formula as described by McGonigle et al. (1990). *A. annua* L. plants cocultivated with *P. indica* resulted in 50.23% colonization, while dual treated plants have better root colonization of 78.99% by *P. indica* (Arora et al. 2016).

Table 15.1 Effect of *P. indica* and *A. chroococcum* alone or in combination on plant growth

Parameters	Control	<i>P. indica</i>	<i>A. chroococcum</i>	<i>P. indica</i> + <i>A. chroococcum</i>
Plant height	60.4 ± 3.36 ^a	79.37 ± 2.76 ^b	74.74 ± 4.42 ^b	98.76 ± 2.68 ^c
Plant biomass	57.71 ± 3.23 ^a	76.14 ± 2.47 ^b	64.84 ± 3.56 ^b	88.07 ± 4.53 ^c
Leaf yield	7.93 ± 1.26 ^a	12.13 ± 1.03 ^b	10.04 ± 1.05 ^b	14.25 ± 1.14 ^c

Plants were grown with *P. indica*, *A. chroococcum*, both *P. indica* + *A. chroococcum*, and control plant without *P. indica* or *A. chroococcum*. Values are presented as means ($n = 8$) ± SD. Different letters (a,b,c) indicate significant differences between each treatment ($P \leq 0.05$) by Tukey's post hoc test

15.3 Effect of *P. indica* and *A. chroococcum* on Nitrogen and Phosphorus

Phosphorus and nitrogen are the important macromolecules that are responsible for increased growth, yield, and quality of plant. Concentrations of phosphorus and nitrogen were significantly higher in those plants cocultivated with dual treatment (Fig. 15.1). On individual basis, plants treated with *P. indica* significantly increased P content by 65.95% and with *A. chroococcum* resulted in 31.90% higher P content in *A. annua* L. plants compared to the control plants, respectively. Likewise, plants treated with *P. indica* significantly increased N content by 13.27% and with *A. chroococcum* resulted in 29.20% higher N content in *A. annua* L. plants compared to the control plants, respectively. The colonization of *A. annua* L. with dual treatment resulted in 86% increase in P content and 55.75% increase in N content (Fig. 15.1). *P. indica* is known to enhance phosphorous uptake in plants, which in turn might enable more energy available for nitrogen fixation by *A. chroococcum*; this could be the reason for higher P and N content in dual treated plants (Arora et al. 2016).

15.4 Effect of *P. indica* and *A. chroococcum* on Chlorophyll Content

Chl a, chl b, and total chlorophyll content was quantified in leaves of *A. annua* L. and found significantly increased in plants treated with *P. indica*, *A. chroococcum* alone, or in combination as compared to the control plants. Chl a showed values of 4.5 and 4.7 mg/g, respectively, for plant treated with *A. chroococcum* and *P. indica*, separately, and 5.6 mg/g fresh weight for plant dual treated with *P. indica* and *A. chroococcum* together. Similarly, the content of chl b exhibited values of 0.7

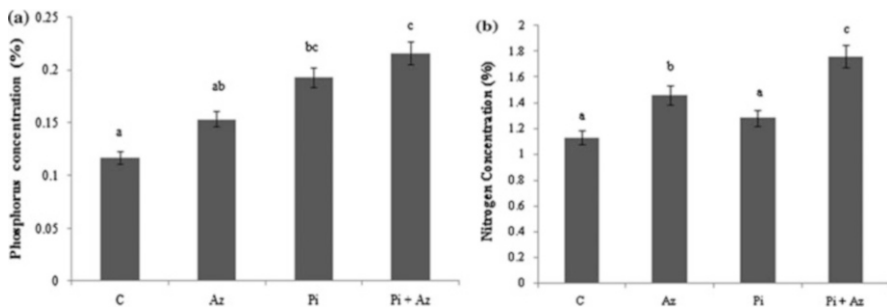


Fig. 15.1 Phosphorus (a) and nitrogen (b) concentration (%) in leaves of *A. annua* L. plants, grown for 2 months after transplanting, under poly house conditions. Columns with different letters are indicating significant differences between each treatment at 5% probability level according to Tukey's post hoc test, and the error bars represent the standard error

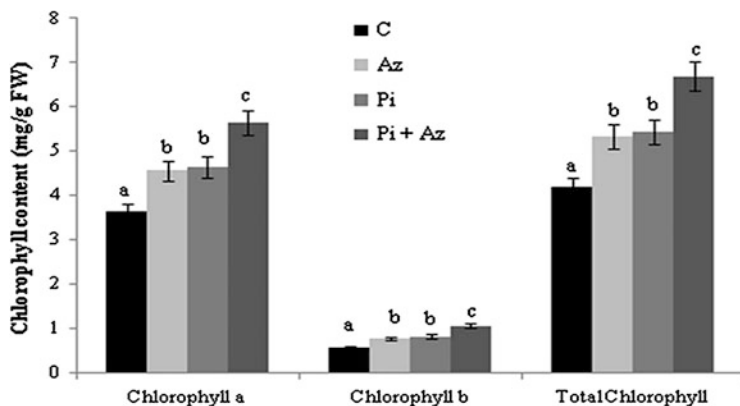


Fig. 15.2 Chlorophyll content (mg/g fresh weight) in leaves of *A. annua* L. plants, grown for 2 months after transplanting, under poly house conditions. Columns with different letters are indicating significant differences between each treatment at 5% probability level according to Tukey's post hoc test, and the error bars represent the standard error

and 0.8 mg/g, respectively, for plant treated with *A. chroococcum* and *P. indica*, separately, and 1.0 mg/g fresh weight for plant dual treated with *P. indica* and *A. chroococcum* together. The plants dual treated with *P. indica* and *A. chroococcum* together also enhanced total chlorophyll content by 57.91% than control plants (Fig. 15.2). However, the chlorophyll content of *A. annua* L. plants treated with *P. indica* and *A. chroococcum*, separately, was not significantly different. More chlorophyll content in the plants is attributed to the fact that an increase in plant nutrition by an increase in P and N uptake will optimize the rate of photosynthesis by increasing the amount of plant chlorophyll, which will lead to an increase in biomass production by C fixation from CO₂. Nitrogen is part of the chlorophyll molecule, which gives green color to plants and is involved in creating food for the plant through photosynthesis.

15.5 Effect of *P. indica* and *A. croococcum* on Artemisinin Content

One gram of dry leaf material was used for the estimation of artemisinin using the method as described by Zhao and Zeng (1986). Derivatized artemisinin was analyzed and quantified through reverse phase column (C18, 5 μ m, 4.6 \times 250 mm) using premix methanol: 100 mM K-phosphate buffer, pH, 6.5 (60:40), as mobile phase at constant flow rate of 1 ml min⁻¹ with the detector set at 260 nm. Artemisinin was quantified with the help of standard curve prepared by HPLC (Fig. 15.3). An overlay of the results obtained with comparative HPLC of a

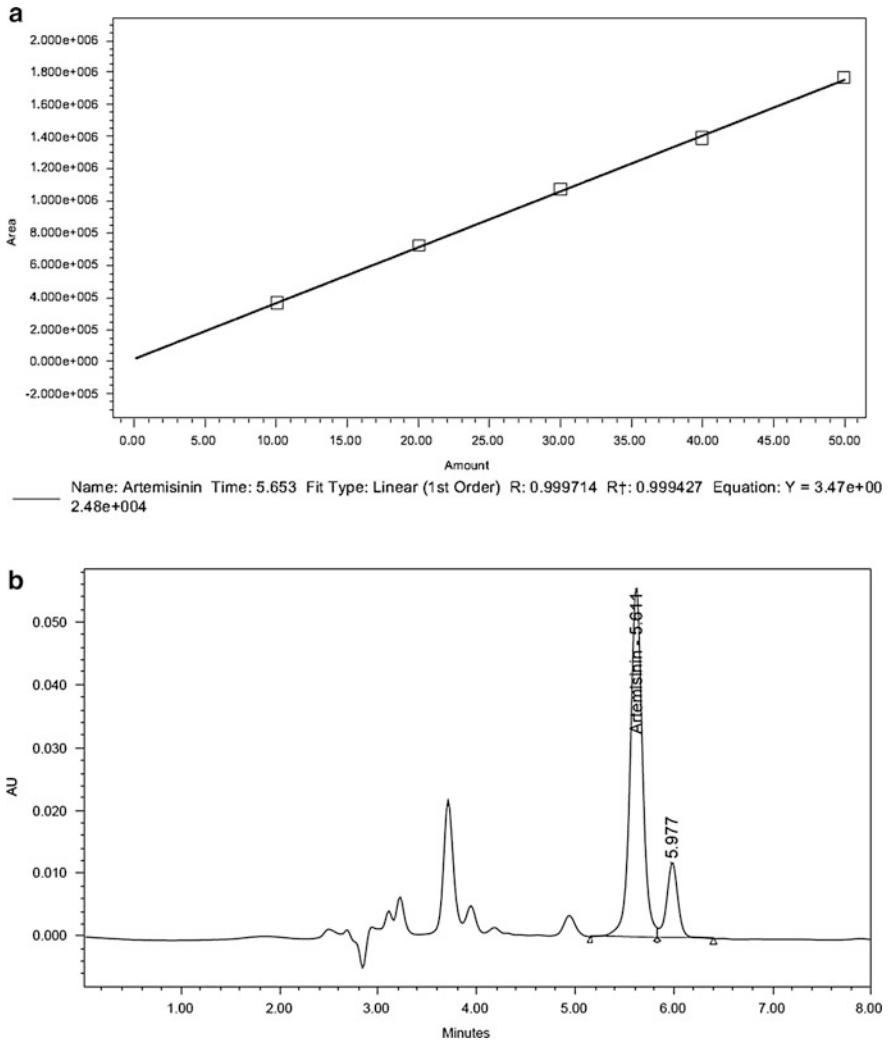


Fig. 15.3 (a) Calibration curve of artemisinin standard. (b) Chromatogram of a standard solution of artemisinin after process prior to analysis (RT = 5.611)

standard solution of artemisinin prior to analysis of samples is shown in Fig. 15.3. Artemisinin content was expressed as % as well as mg g^{-1} dw of leaves.

The symbiotic effectiveness was much evident when artemisinin content was recorded 70% higher in *A. annua* L. plants subjected to dual inoculation (Fig. 15.4). *P. indica* colonization or *A. croococcum* inoculation independently enhanced artemisinin content to approximately similar levels. The enhanced concentration of artemisinin by dual treatment may be due to improved growth and nutrient status of the plants (Arora et al. 2016; Davies et al. 2009).

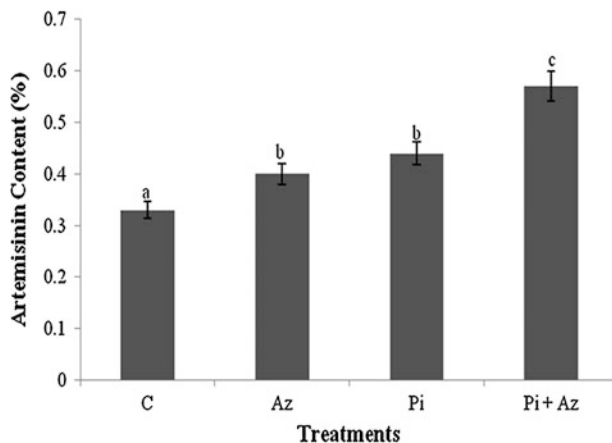


Fig. 15.4 Artemisinin content (%) in leaves of *A. annua* L. plants, grown for 2 months after transplanting, under poly house conditions. Columns with different letters are indicating significant differences between each treatment at 5% probability level according to Tukey's post hoc test, and the error bars represent the standard error

15.6 Conclusion

Interaction of *A. annua* L. with both *P. indica* and *A. chroococcum* in cocultivation resulted in improved plant biomass and concentration of artemisinin in the plant as compared to control and singly treated plants. The combinatorial application of *P. indica* with *A. chroococcum* induces reprogramming of many cellular activities like phytohormone biosynthesis, nutrient acquisition, and secondary metabolite synthesis in *A. annua* L. leading to higher biomass and enhanced artemisinin content and yield. The use of this microbial consortium as bio-fertilizer in place of chemical fertilizers, hence, presents a viable option for increased artemisinin availability.

15.7 Future Prospects

The current study provides a perspective into study of combined inoculation of symbiotic fungus and nitrogen-fixing bacteria and their interaction with plants. Different beneficial and symbiotic bacterial fungal associations can also be studied with plants to check their effect on plant yield, disease resistance, abiotic and biotic stress response, production of important molecules, and plant products. It will also help to understand the molecular mechanism between the microorganisms and determine the active compounds released that help in plant trait enhancement. Proteomic studies can also be carried out to check the effect of consortium on plants. Hence, this consortium can also be used to check their effect on other plant

species. Further study is also required to check the effectiveness of microbial consortia in making the plant resistant to pathogens through systemic induced resistance.

References

- Abdin MZ, Israr M, Rehman RU, Jain SK (2003) Artemisinin, a novel antimalarial drug: biochemical and molecular approaches for enhanced production. *Planta Med* 69:289–299
- Arora M, Saxena P, Choudhary DK, Abdin MZ, Varma A (2016) Dual symbiosis between *Piriformospora indica* and *Azotobacter chroococcum* enhances the artemisinin content in *Artemisia annua* L. *World J Microbiol Biotechnol* 32:19. doi:[10.1007/s11274-015-1972-5](https://doi.org/10.1007/s11274-015-1972-5)
- Awasthi A, Bharti N, Nair P et al (2011) Synergistic effect of *Glomus mosseae* and nitrogen fixing *Bacillus subtilis* strain Daz26 on artemisinin content in *Artemisia annua* L. *Appl Soil Ecol* 49:125–130
- Bakker PAHM, Berendsen RL, Doornbos RF, Wiermans PCA, Pieterse CMJ (2013) The rhizosphere revisited: root microbiomics. *Front Plant Sci* 4:165. doi:[10.3389/fpls.2013.00165](https://doi.org/10.3389/fpls.2013.00165)
- Bandyopadhyay P, Bhuyan S, Yadava PK, Varma A (2016a) Soluble factors from *Azotobacter chroococcum* modulate growth of *Piriformospora indica* in co-cultures. *Endocytobiosis Cell Res* 27:9–13
- Bandyopadhyay P, Bhuyan SK, Yadava PK, Varma A, Tuteja N (2016b) Emergence of plant and rhizospheric microbiota as stable interactomes. *Protoplasma* 254:617–626. doi:[10.1007/s00709-016-1003-x](https://doi.org/10.1007/s00709-016-1003-x)
- Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci* 17:478–486
- Bhuyan SK, Bandyopadhyay P, Kumar P, Mishra DK, Prasad R et al (2015) Interaction of *Piriformospora indica* with *Azotobacter chroococcum*. *Sci Rep* 5:13911. doi:[10.1038/srep13911](https://doi.org/10.1038/srep13911)
- Chaudhary V, Kapoor R, Bhatnagar AK (2008) Effectiveness of two arbuscular mycorrhizal fungi on concentrations of essential oil and artemisinin in three accessions of *Artemisia annua* L. *Appl Soil Ecol* 40:174–181. doi:[10.1016/j.apsoil.2008.04.003](https://doi.org/10.1016/j.apsoil.2008.04.003)
- Davies MJ, Atkinson CJ, Burns C et al (2009) Enhancement of artemisinin concentration and yield in response to optimization of nitrogen and potassium supply to *Artemisia annua*. *Ann Bot* 104:315–323
- Javot H, Pumplin N, Harrison MJ (2007) Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant Cell Environ* 30:310–322
- Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil Soils* 37:1–16
- Kapoor R, Chaudhary V, Bhatnagar AK (2007) Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* 17:581–587
- Lakshminarayana K, Narula N, Hooda IS, Faroda AS (1992) Nitrogen economy in wheat (*Triticum aestivum*) through use of *Azotobacter chroococcum*. *Ind J Agric Sci* 62:75–76
- Mandal S, Upadhyay S, Wajid S et al (2015) Arbuscular mycorrhiza increase artemisinin accumulation in *Artemisia annua* by higher expression of key biosynthesis genes via enhanced jasmonic acid levels. *Mycorrhiza* 25(5):345–357. doi:[10.1007/s00572-014-0614-3](https://doi.org/10.1007/s00572-014-0614-3)
- Martyniuk S, Martyniuk M (2003) Occurrence of *Azotobacter* spp. in some polish soils. *Pol J Environ Stud* 12:371–374
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 115:495–501

- Miller LH, Su X (2011) Artemisinin: discovery from the Chinese herbal garden. *Cell* 146:855–858
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 5:158–161
- Pozo MJ, Jung SC, Lopez-Raez JA, Azcon-Aguilar C (2010) Impact of Arbuscular Mycorrhizal Symbiosis on plant response to biotic stress: the role of plant defence mechanisms. In: Koltai H, Kapulnik Y (eds) *Arbuscular mycorrhizas: physiology and function*, vol 193. Springer Science+Business Media B.V., Dordrecht, pp 193–207. doi:[10.1007/978-90-481-9489-6_9](https://doi.org/10.1007/978-90-481-9489-6_9)
- Prasad R, Kumar M and Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) *Plant growth-promoting rhizobacteria and medicinal plants*. Springer International Publishing, pp 247–260
- Rapparini F, Llusia J, Penuelas J (2008) Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of *Artemisia annua* L. *Plant Biol* 10:108–122
- Romero MR, Efferth T, Serrano MA et al (2005) Effect of artemisinin/artesunate as inhibitors of hepatitis B virus production in an ‘in vitro’ replicative system. *Antiviral Res* 68(2):75–83
- Sharma G, Agrawal V (2013) Marked enhancement in the artemisinin content and biomass productivity in *Artemisia annua* L. shoots co-cultivated with *Piriformospora indica*. *World J Microbiol Biotechnol* 29:1133–1138
- Singh NP, Lai HC (2004) Artemisinin induces apoptosis in human cancer cells. *Anticancer Res* 24:2277–2280
- Vafadar F, Amooaghaie R, Otrushy M (2014) Effects of plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *J Plant Interact* 9:128–136. doi:[10.1080/17429145.2013.779035](https://doi.org/10.1080/17429145.2013.779035)
- Walker V, Couillerot O, Felten AV, Bellvert F et al (2012) Variation of secondary metabolite levels in maize seedling roots induced by inoculation with *Azospirillum*, *Pseudomonas* and *Glomus* consortium under field conditions. *Plant Soil* 356:151–163
- Willcox M, Bodeker G, Bourdy G et al (2004) *Artemisia annua* as a traditional herbal antimalarial. In: Wilcox ML, Bodeker G, Rasoanaivo P, Addae-Kyereme J (eds) *Traditional medicinal plants and malaria*. CRC Press, Boca Raton, IL
- Zhao SS, Zeng MY (1986) Determination of Qinghaosu in *Artemisia annua* L. by high performance liquid chromatography. *Chinese J Pharma Anal* 6:3–5

Chapter 16

Microbial Symbiosis and Bioactive Ingredients of Medicinal Plants

Divya Kilam, Priyanka Sharma, Abha Agnihotri, Amit Kharkwal,
and Ajit Varma

Abstract Medicinal plants have been used widely for their bioactive ingredients as they are highly potent and have least side effects. This has led to a surge in demand for medicinal plants for producing higher quantity and good quality bioactive compounds. Symbiotic association of microorganisms with plants has been shown to affect the production and quality of active ingredients. However, the effect is not consistent and is seen to vary under different microbial associations. This chapter elucidates the studies on microbial symbiosis with medicinal plants and the effect of this interaction on medicinally important bioactive ingredients. The role of both nutritional and non-nutritional pathways in this interaction has also been discussed.

16.1 Introduction

Plants have been used as an important source of medicine in pharmaceutical biology since thousands of years. As per WHO estimates, even today, up to 80% of population rely on traditional medicines for primary healthcare needs (Sieniawska et al. 2013). Medicinal plants are a rich source of bioactive compounds, which are widely used as potent drugs for their therapeutic properties (Gu et al. 2014). They are also used in pharmaceuticals, food additives, fragrances and industrially important compounds. The use of medicinal plants for the cure of ailments has a long history in cultures across the globe. The Chinese book on roots and grasses, written around 2500 BC, mentioned 365 drugs from dried parts of medicinal plants (Bottcher 1965). The Indian Vedas also mention about treatment with plants and their products (Tucakov 1971). The Ebers Papyrus, written around circa 1550 BC, refers to 700 plant species and drugs used for therapy (Glesinger 1954). About 7000 species of medicinal plants have been reported in China alone (“Center for Traditional Medicinal Plants,” 2016). In India, around 25,000 effective plant-based

D. Kilam • P. Sharma • A. Agnihotri (✉) • A. Kharkwal • A. Varma
Amity Institute of Microbial Technology, Amity University, Block E-3, 4th Floor, Sector 125,
Noida 201303, Uttar Pradesh, India
e-mail: aagnihotri@amity.edu

formulations are used in traditional medicines to cure different diseases (Pandey et al. 2013). Natural medicines have gained popularity among consumers as they are effective and safe to use and have lesser side effects. This increasing demand for plant-based medicines has resulted in large-scale production of medicinal plants using modern techniques. Innovative strategies are also required to maintain the quality of medicinal plant products as they get affected due to pests, diseases and excessive use of pesticides.

Plant growth and development is synergistic combination of a number of environmental factors. Plants being the motionless entities are confronted with a number of unfavourable conditions, e.g. salinity, drought, pathogen attacks, etc. However, with the course of evolution, they have developed a number of mechanisms to protect themselves from the attack of such stresses (Kogel et al. 2006). They produce bioactive compounds as a survival mechanism against biotic and abiotic stresses (Großkinsky et al. 2016). These stress reactions are triggered by elicitors that can be abiotic, such as metal ion or inorganic compounds or biotic, derived from a biological source or their products. Studies have shown that treatment of plants with biotic elicitor can be employed for the production of plant secondary metabolites (Gorelick and Bernstein 2014). This can lead to activation of defence reactions which would cause production of bioactive molecules like phytoalexins in the plants. Elicitation is thus being used to induce production of secondary metabolites in plants. The three major groups of plant secondary metabolites, terpenoids, phenolics and alkaloids, are used in preparation of medicinal products. Essential oils from medicinal plants, mostly consisting of monoterpenes, sesquiterpenes and phenylpropanoids, are used as flavours, fragrances, antioxidants and antimicrobial agents (Guenther 2013). The study of interaction of microbial diversity with medicinal plants is thus important as it will help determine their impact on bioactive compounds and also help improve the quality of the produce. The best strategy adapted by plants is to form a mutualistic association with beneficial microorganisms to protect themselves from stresses (Lum and Hirsch 2003). However, the most difficult task is to distinguish between mutualistic partners and parasites (Kogel et al. 2006; Schulz and Boyle 2005), because both of these interactions share a number of common signalling pathways (Paszkowski 2006). This chapter gives an account of the studies on accumulation of plant bioactive compounds in a variety of medicinal plants upon association with beneficial microorganisms and the proven or putative mechanisms by which these microbes promote the production of bioactive compounds in plants.

16.2 Bioactive Constituents of Medicinal Plants

The damaging effects of all environmental stresses on plants can be observed as either death of the plant or decrease in productivity. The response generated by plant correlates well with that of oxidative stress. Oxidative stress results in the generation of free oxygen radicals or reactive oxygen species (ROS), which are

cytotoxic at high concentrations (McKersie 1996). These radicals catalyse self-propagating autoxidation reactions that lead to formation of other organic peroxides, which cause major damage to biological system. Various cellular locations and the environments where the free oxygen species are formed demand for a scavenging system for uninterrupted growth and survival of plants (Nath et al. 2016). To fight against the deleterious effects of reactive oxygen species, plants are endowed with several antioxidants and metabolites in different plant cell compartments (Ashraf and Harris 2004). These active compounds are mainly secondary metabolites or their derivatives like alkaloids, glycosides, terpenes, flavonoids, tannins, phenolics, anthraquinones, saponins and essential oils (Croteau et al. 2000; Bagde et al. 2010). More than 12,000 alkaloids are known to be present in 20% of plant species, and over 4000 flavonoids are reported in nearly 70% of plant species, which suggests the vastness of these compounds (Heim et al. 2002; Ziegler and Facchini 2008). Essential oils or volatile oils are also present in medicinal plants and are known to comprise of more than 200 different chemical components (Martinez et al. 2008). Medicinal plants, in particular, have been exploited by humans for this pool of phytochemicals. Thus the primary focus of research on medicinal plants has been on the bioactive compounds with therapeutic properties.

16.3 Microorganisms for Plant Secondary Metabolite Enhancement

Soil microflora consists of a range of microorganisms, such as algae, bacteria and fungi. These microorganisms contribute actively in almost all the chemical processes that occur within the soil. They participate in carbon and nitrogen cycling, nutrient acquisition, tolerance to various stresses and other processes that are important for the survival and growth of the plant. In contrast, plants can have enormous effects on soil microbial communities especially those colonizing the rhizospheric region. This is because of increasing the availability of carbon in the soil due to the release of root exudates and decaying plant material, which will act as growth substrate, structural material or signal for them (Barea et al. 2005).

16.3.1 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are known to form symbiotic association with more than 80% of vascular plants on the earth. They are found under all climates and in all ecosystems, regardless of the type of soil, vegetation or growing conditions. AM fungi colonize the roots and rhizosphere, and the hyphae being thinner branch more frequently than plant roots and spread out over several centimetres in the form of ramified filaments. This extended network increases the absorptive

capacity of roots and allows the plant to have better access to a greater quantity of water and minerals required for nutrition. Their association thus increases water uptake and availability of nutrients to the plant, especially insoluble soil phosphate (Clark and Zeto 2000), and the fungus in turn is benefitted by supply of carbohydrates derived from plant photosynthesis (Harrison 1999; Gianinazzi et al. 2010). The fungi provide nourishment, increase the reproductive potential, improve root performance and provide a natural defence against invaders, like pests and pathogens (Singh et al. 2000). The use of AMF was proposed for a number of medicinal plant species (Toussaint et al. 2007; Jurkiewicz et al. 2010; Copetta et al. 2006), endangered plants (Sharma et al. 2007; Bothe et al. 2010) and for the restoration of devastated habitats (Turnau and Haselwandter 2002).

The potential of AMF to promote accumulation of bioactive compounds in medicinal plants has been investigated by several researchers. The first studies of AMF affecting medicinal plants were conducted by Honggang (1989). They studied the effect of *Glomus mosseae* and *Glomus epigaeum* on the growth, nutrient uptake and synthesis of effective compounds, hyoscyne in medicinal herb *Datura stramonium* L. The hyoscyne content was found to increase significantly by 103.2–117.2% (Honggang 1989). Another study by the same group showed enhanced growth, nutrient uptake and volatile oil synthesis in *Schizonepeta tenuifolia* upon inoculation with AMF, wherein the volatile oil content increased by 163.6–209.1% as compared to control (Wei and Wang 1991). Since then symbiosis between many different medicinal plants and AMF has been studied for accumulation of important bioactive compounds (Table 16.1). AM symbiosis has also shown to change the composition of bioactive compounds in medicinal plants. AM symbiosis in *Salvia officinalis* showed changes in the composition of essential oils with enhanced quantities of bornyl acetate, 1,8-cineole, α -thujones and β -thujones (Geneva et al. 2010). The composition of essential oils in *Origanum onites* and *Mentha viridis* was also seen to differ upon mycorrhization (Karagiannidis et al. 2011). Contrasting results have also been seen in some of the studies, with no change in the chemical composition of these bioactive compounds upon inoculation with AMF. *Origanum vulgare* plants inoculated with AMF showed a similar chemical profile of essential oils as seen in control plants (Morone Fortunato and Avato 2008). Studies by Lermen et al. (2015) showed similar results with no significant change in composition of essential oils in *Cymbopogon citratus* upon mycorrhization.

16.3.2 Root Endophytic Fungus

Another type of symbiotic relationship that the plants form is with the endophytes. The non-mycorrhizal microbes such as dark septate endophyte, *Phialocephala fortinii*, *Cryptosporiopsis* spp., *Piriformospora indica*, *Fusarium* spp. and *Cladorrhinum foecundissimum* have been shown to improve the growth of their hosts after colonization (Schulz 2006; Chadha et al. 2014). Unlike mycorrhizal fungi, endophytes are known to reside and grow within plant tissues, leaves, bark, stems and roots (Carroll 1988). Root endophytes inhabit the roots without forming the

Table 16.1 Effect of inoculation of *Arbuscular mycorrhizal* fungi on secondary metabolite content of medicinal plants

Medicinal plant	Arbuscular mycorrhizal fungi	Secondary metabolite content	Reference
<i>Anadenanthera colubrina</i>	<i>Glomus</i> spp.	Enhanced phenol, flavonoids and total tannins	Pedone-Bonfim et al. (2013)
<i>Anethum graveolens</i>	<i>Glomus macrocarpum</i> , <i>Glomus fasciculatum</i>	Essential oil increased up to 90%	Kapoor et al. (2002)
<i>Angelica archangelica</i> L.	<i>Glomus intraradices</i> , <i>Glomus mosseae</i>	Enhanced monoterpenoids and coumarins	Zitterl-Eglseer et al. (2015)
<i>A. dahurica</i>	<i>Glomus</i> spp.	Increased total coumarin content	Zhao et al. (2009), Zhao and He (2011)
<i>Arnica montana</i>	<i>G. intraradices</i>	Increased secondary metabolite content	Jurkiewicz et al. (2010)
<i>A. montana</i>	<i>G. intraradices</i>	Increased phenolic acids in roots	Jurkiewicz et al. (2010)
<i>Artemisia annua</i> L.	<i>G. macrocarpum</i> , <i>G. fasciculatum</i>	Enhanced artemisinin and essential oil content	Kapoor et al. (2007), Chaudhary et al. (2008)
<i>A. annua</i>	<i>Rhizophagus intraradices</i>	Enhanced artemisinin content	Mandal et al. (2015)
<i>Bupleurum scorzoniferifolium</i>	<i>G. mosseae</i>	Enhanced flavonoid content	Teng and He (2005)
<i>Camptotheca acuminata</i>	<i>G. intraradices</i>	Increased camptothecin content	Zhao et al. (2006)
<i>Castanospermum australe</i>	<i>Glomus</i> spp.	Increased castanospermine content	Abu-Zeyad et al. (1999)
<i>Catharanthus roseus</i>	<i>Glomus</i> spp.	Increased phenolic compound and vinblastine content	De la Rosa-Mera et al. (2011)
<i>Coleus forskohlii</i>	<i>Glomus bagyarajii</i>	Increased forskolin content	Sailo and Bagyaraj (2005)
<i>Cymbopogon citratus</i> S.	<i>Rhizophagus clarus</i>	Increased essential oil content	Lermen et al. (2015)
<i>Echinacea purpurea</i>	<i>G. intraradices</i>	Enhanced phenolics in roots	Araim et al. (2009)
<i>Foeniculum vulgare</i>	<i>G. macrocarpum</i> and <i>G. fasciculatum</i>	Essential oil increased by 78%	Kapoor et al. (2004)
<i>Glycyrrhiza uralensis</i>	<i>G. mosseae</i> , <i>G. versiforme</i>	Enhanced glycyrrhizin concentration	Liu et al. (2007)
<i>Hypericum perforatum</i>	<i>G. intraradices</i> , <i>G. mosseae</i> , <i>G. constrictum</i> , <i>G. geosporum</i>	Higher hypericin and pseudohypericin content	Zubek et al. (2012)

(continued)

Table 16.1 (continued)

Medicinal plant	Arbuscular mycorrhizal fungi	Secondary metabolite content	Reference
<i>Inula ensifolia</i>	<i>Glomus clarum</i>	Increased thymol derivative content	Zubek et al. (2010)
<i>Mentha arvensis</i>	<i>G. fasciculatum</i>	Significantly increased oil content and oil yield	Gupta et al. (2002)
<i>M. crispata</i>	<i>Glomus etunicatum</i> , <i>R. clarus</i>	Enhanced essential oil content	Urcoviche et al. (2015)
<i>M. viridis</i>	<i>G. etunicatum</i> , <i>Glomus lamellosum</i>	High levels (>5%) of limonene, 1,8-cineole, linalool, carvone, eugenol, (E)-methyl cinnamate	Karagiannidis et al. (2011)
<i>Ocimum basilicum</i>	<i>G. intraradices</i>	Enhanced anthocyanin concentration	Lee and Scagel (2009)
<i>Origanum onites</i>	<i>G. etunicatum</i> , <i>G. lamellosum</i>	High levels (>5%) of sabinene, terpinene, trans-sabinene hydrate, terpinen-4-ol, carvacrol	Karagiannidis et al. (2011)
<i>Origanum</i> sp.	<i>G. mosseae</i>	Increased essential oil concentration	Copetta et al. (2006), Khaosaad et al. (2006)
<i>Passiflora alata</i> C.	<i>Gigaspora albida</i>	Increased flavonoids	Oliveira et al. (2015)
<i>Phellodendron amurense</i>	<i>G. mosseae</i> , <i>G. etunicatum</i> , <i>G. versiforme</i> , <i>G. diaphanum</i>	Increased berberine, jatrorrhizine, palmatine content	Fan et al. (2006)
<i>Phellodendron chinense</i>	<i>Acaulospora laevis</i> , <i>Acaulospora mellea</i> , <i>Glomus ditum</i> , <i>G. intraradices</i> , <i>G. mosseae</i> , <i>G. versiforme</i>	Increased berberine content	Zhong and Fan (2007)
<i>Pinellia ternata</i>	<i>G. mosseae</i> , <i>G. intraradices</i>	Increased guanosine and alkaloid content	Guo et al. (2010)
<i>Pogostemon cablin</i>	<i>Acaulospora laevis</i> , <i>Gigaspora margarita</i> , <i>G. bagyarajii</i> , <i>G. etunicatum</i> , <i>G. fasciculatum</i> , <i>G. intraradices</i> , <i>G. leptotichum</i> , <i>G. macrocarpum</i> , <i>G. monosporum</i> , <i>G. mosseae</i> , <i>S. calospora</i>	Enhanced essential oil content	Arpana et al. (2008)
<i>Prosopis laevigata</i>	<i>G. rosea</i>	Increased trigonelline content	Rojas-Andrade et al. (2003)
<i>Salvia miltiorrhiza</i>	<i>G. mosseae</i>	Enhanced essential oil content	Meng and He (2011)

(continued)

Table 16.1 (continued)

Medicinal plant	Arbuscular mycorrhizal fungi	Secondary metabolite content	Reference
<i>S. officinalis</i>	<i>G. intraradices</i>	Enhanced essential oils, 1,8-cineole, α - and β -thujones	Geneva et al. (2010)
<i>S. officinalis</i>	<i>G. intraradices</i> , <i>G. mosseae</i>	Enhanced phenolic and rosmarinic acid content	Nell et al. (2009)
<i>Stevia rebaudiana</i>	<i>R. fasciculatus</i>	Increase in steviol glycoside content	Mandal et al. (2013)
<i>Trachyspermum ammi</i>	<i>G. macrocarpum</i> , <i>G. fasciculatum</i>	Essential oil increased by 72%	Kapoor et al. (2002)
<i>Viola tricolor</i> L.	<i>Rhizophagus irregularis</i> , <i>Funneliformis mosseae</i>	Increased phenolic acid and flavonoid concentration	Zubek et al. (2015)

typical anatomical features of mycorrhiza and without showing the signs of pathogenesis. The fungal root endophytes involve a diverse group of fungi. The most-studied taxa have dark pigmented hyphal walls and are referred as the dark septate endophytes (DSE). They are morphologically defined fungi belonging to the group of ascomycetes. They are frequently observed in cortex, epidermis as well as the root surface (Knapp et al. 2015). Colonization by DSE has shown to increase growth of the plants (Mayerhofer et al. 2013). *Trichoderma* is another true endophyte which is mainly found in the root ecosystems. In the last few decades, there has been a spurt of research focused on plant-endophyte associations and their application as a potential bio augmenting agent. Besides imparting beneficial effects on plant growth and health, the association also provides tolerance against various biotic and abiotic stresses (Hermosa et al. 2012). Another well-studied group of the root endophytes are the Sebaciniales. *Piriformospora indica* is an extensively studied member of this group. *P. indica* is able to associate itself with roots of various plant species in a manner similar to AM fungi (Varma et al. 1999, 2001; Sharma et al. 2014; Das et al. 2012, 2013; Singh et al. 2003). *P. indica* promotes nutrient uptake and enhances the growth and biomass of the plant, including monocots and dicots (Yadav et al. 2010; Varma et al. 2000, 2012), induces early flowering (Das et al. 2012, 2013), increases the resistance against fungal pathogens and allows the plant to survive under stressed environment (Das et al. 2012; Harman 2011; Gill et al. 2016). It has been established as a bio fertilizer, bio protectant and biological hardening agent (Johnson et al. 2014; Varma et al. 2012). Sharma et al. (2014) reported higher antioxidant activity and greater secondary metabolite content in *P. indica*-colonized *A. vera* plantlets thus enhanced ability to reduce oxidative stress and fight against various phytopathogenic diseases. The phylogenetic relationship of another new species, *Piriformospora williamsii* with Sebaciniales, has been studied by Basiewicz et al. (2012). The study also showed biphasic lifestyle of mutualistic symbiont *P. indica*. Researchers have endeavoured to know the molecular mechanisms during plant-endophyte association and their effect on plants. However, only few documents refer

Table 16.2 Effect of inoculation of root endophytic fungi on secondary metabolite content of medicinal plants

Root endophytic fungus	Medicinal plant	Secondary metabolite content	Reference
Dark septate endophytic (DSE) fungi (seven isolated strains)	<i>Epimedium wushanense</i>	One of the strains (DSE8) showed improved flavonoid and icariin content	Zhu et al. (2015)
<i>Piriformospora indica</i>	<i>Aloe vera</i> L.	Increased aloin content and anti-oxidant activity	Sharma et al. (2014)
	<i>Aristolochia elegans</i>	Enhancement in aristolochic acid	Bagde et al. (2014)
	<i>Artemisia annua</i>	Enhancement in artemisinin content	Sharma and Agrawal (2013)
	<i>Bacopa monnieri</i>	Higher bacoside content	Prasad et al. (2013)
	<i>Centella asiatica</i>	Enhanced asiaticoside content	Satheesan et al. (2012)
	<i>Coleus forskohlii</i>	Enhancement in essential oils (p-cymene, nonanal)	Das et al. (2012)
	<i>Curcuma longa</i>	Increased curcumin content	Bajaj et al. (2014)
	<i>Linum album</i>	Increase in podophyllotoxin and 6-methoxypodophyllotoxin	Baldi et al. (2008)
<i>Withania somnifera</i>	Increased withaferin A content	Ahlawat et al. (2016)	

secondary metabolite accumulation in plants upon interaction with fungal root endophytes (Table 16.2).

16.3.3 Plant Growth-Promoting Rhizobacteria

Plant growth-promoting rhizobacteria (PGPRs) are a specific group of soil bacteria that colonize the rhizosphere and rhizoplane of plants. They are known to enhance plant growth through mechanisms like nitrogen fixation, phosphate solubilization and quorum sensing (Prasad et al. 2015; Goswami et al. 2016). Rhizobacteria have the ability to successfully colonize the plant roots and positively enhance plant growth. They can also improve the secondary metabolite content in plants by improving the phosphorus status or by altering the hormonal balance of the plants (Köberl et al. 2015). Till date, several studies have reported the ability of PGPRs to promote growth of cereals, vegetable and food crops. However, there are limited reports on interaction of PGPRs with medicinal plants for accumulation of secondary metabolites, as summarized in Table 16.3.

Table 16.3 Effect of inoculation of plant growth-promoting rhizobacteria on secondary metabolite content of medicinal plants

Medicinal plant	PGPR	Secondary metabolite content	Reference
<i>Anoectochilus roxburghii</i>	<i>B. subtilis</i>	Increase in flavonoids and essential oil content	Refish et al. (2016)
<i>Bacopa monnieri</i>	<i>Bacillus pumilus</i> , <i>Exiguobacterium oxidotolerans</i>	Increase in bacoside-A content	Bharti et al. (2013)
<i>Catharanthus roseus</i>	<i>Pseudomonas fluorescens</i>	Increased production of ajmalicine	Jaleel et al. (2007)
<i>C. roseus</i>	<i>Azotobacter chroococcum</i> , <i>P. fluorescens</i> , <i>Bacillus megaterium</i>	Enhanced alkaloid content	Karthikeyan et al. (2010)
<i>C. roseus</i> varieties 'rosea' and 'alba'	<i>Azospirillum brasilense</i> , <i>P. fluorescens</i>	Enhanced ajmalicine content	Karthikeyan et al. (2009)
<i>Glycine max</i>	<i>P. fluorescens</i>	Enhanced isoflavone content	Algar et al. (2012)
<i>Hyoscyamus niger</i> L.	<i>Pseudomonas putida</i> , <i>P. fluorescens</i>	Increase in tropane alkaloids; hyoscyamine, scopolamine	Ghorbanpour et al. (2011, 2013)
<i>Matricaria chamomilla</i> L.	<i>Azospirillum lipoferum</i> , <i>A. chroococcum</i>	Increased essential oil content	Dastborhan et al. (2011)
<i>Mentha piperita</i>	<i>P. fluorescens</i> , <i>B. subtilis</i> , <i>A. brasilense</i>	Increased essential oil (pulegone, menthone) content	Santoro et al. (2011)
<i>M. piperita</i>	<i>B. subtilis</i> , <i>P. fluorescens</i> , <i>P. putida</i>	Marked changes in monoterpene accumulation	Del Rosario et al. (2015)
<i>Ocimum basilicum</i>	<i>B. subtilis</i>	Increase in essential oil (α -terpineol and eugenol) content	Banchio et al. (2009)
<i>Origanum majorana</i> L.	<i>P. fluorescens</i> , <i>B. subtilis</i> , <i>Sinorhizobium meliloti</i> , <i>Bradyrhizobium</i> sp.	Increased essential oil content	Banchio et al. (2008)
<i>Salvia miltiorrhiza</i> B.	<i>Bacillus cereus</i>	Increased accumulation of tanshinone	Zhao et al. (2010)
<i>S. officinalis</i> L.	<i>P. putida</i> , <i>P. fluorescens</i>	Increased essential oils content	Ghorbanpour et al. (2014)
<i>Tagetes minuta</i>	<i>P. fluorescens</i> , <i>A. brasilense</i>	Enhanced essential oil content	Del Rosario et al. (2013)
<i>Trigonella foenum-graecum</i>	<i>Bacillus</i> sp.	Enhancement in diosgenin levels	Jasim et al. (2015)
<i>Withania somnifera</i>	<i>Azospirillum</i> , <i>A. chroococcum</i> , <i>P. fluorescens</i> , <i>Bacillus megaterium</i>	Enhanced alkaloid content	Rajasekar and Elango (2011)

16.3.4 Microbial Cocultures

Soil microorganisms influence the plant health by increasing the extent of their root system and in turn the ability to acquire nutrients from the soil (Bucio et al. 2007). Engineering the plant rhizosphere through inoculation of specific microorganisms can provide cumulative benefits to the plant. Several beneficial microorganisms have been studied for their potential as a biocontrol agent and are being used as bio fertilizers (Bhardwaj et al. 2014). The study of antagonistic or synergistic effects of different microbial inoculants is however critical for development of an effective host-microbe interaction. Several studies have been undertaken to see the effect of symbiotic fungi and plant growth-promoting rhizobacteria on plant. Toro et al. (1997) reported that dual inoculation of AM fungus, *Glomus intraradices*, and PGPR, *Bacillus subtilis*, increased the plant biomass and tissue phosphorus accumulation. Synergistic effect of *G. intraradices* and *B. subtilis* was also seen in *Lactuca sativa*, where combined inoculations resulted in 77% enhanced plant growth as compared to control plants (Kohler et al. 2007). The potential benefits of dual inoculation of endophytic fungus, *P. indica*, with AM fungi and PGPR (s) have also been reported. Meena et al. (2010) reported that combined inoculations of *P. indica* and *Pseudomonas striata* lead to a significant increase in plant biomass and grain yield of *Cicer arietinum* L. (chickpea). Another study by Nautiyal et al. (2010) showed enhanced nodulation and plant growth promotion in chickpea upon inoculation with *P. indica* and *Paenibacillus lentimorbus*. Recent studies have shown that microbial coculture systems can act as effective tools for biotic elicitation of plant secondary metabolites (Table 16.4).

Green house studies on *Solanum viarum* seedlings showed enhancement in secondary metabolite content, when plants were inoculated with AMF, *Glomus aggregatum*, and PGPR(s), *Bacillus coagulans* and *Trichoderma harzianum* (Hemashenpagam and Selvaraj 2011). Synergistic effect of four different AM fungi, *G. aggregatum*, *Glomus fasciculatum*, *G. intraradices* and *G. mosseae* with PGPR, *B. subtilis*, showed an increase in herb biomass and total oil yield in *Pelargonium graveolens*. The herb yield increased by 59.5% when a combination of *G. mosseae* and *B. subtilis* was used for inoculation experiments (Alam et al. 2011). Endophytic fungus, *P. indica*, is known to form a synergistic association with PGPR, *A. chroococcum*. Studies by Bhuyan et al. (2015) showed that *A. chroococcum* (WR5 and M4 strains) influences the overall growth and physiology of *P. indica* to enter into a symbiotic association. The potential of *P. indica* in combination with *A. chroococcum* (WR5 strain) on secondary metabolite content of *Stevia rebaudiana* has been reported by our laboratory. The study showed a marked increase in total flavonoid and phenolic content in plants treated with dual inoculations of *P. indica* and *A. chroococcum* as compared to singly inoculated and control plants. The major steviol glycosides, stevioside and rebaudioside-A, also showed significant enhancement in plants given combined inoculation (Kilam et al. 2015).

Table 16.4 Effect of inoculation of microbial cocultures on secondary metabolite content of medicinal plants

Microbial coculture	Medicinal plant	Secondary metabolite content	References
AMF + PGPR(s)			
<i>Glomus aggregatum</i> , <i>Bacillus coagulans</i> + <i>Trichoderma harzianum</i>	<i>Solanum viarum</i>	Increase in total phenols, flavonoids, alkaloids, saponins, tannins	Hemashenpagam and Selvaraj (2011)
<i>G. aggregatum</i> , <i>Glomus fasciculatum</i> , <i>G. intraradices</i> , <i>G. mosseae</i> + <i>B. subtilis</i>	<i>Pelargonium graveolens</i>	Increase in the total oil yield	Alam et al. (2011)
<i>G. intraradices</i> + <i>Bacillus polymyxa</i> , <i>P. putida</i> , <i>A. chroococcum</i>	<i>S. rebaudiana</i>	Enhanced stevioside content	Vafadar et al. (2014)
<i>G. mosseae</i> + <i>B. subtilis</i> , <i>P. fluorescens</i>	<i>Thymus daenensis</i>	Increased concentrations of thymol	Bahadori et al. (2013)
<i>Glomus</i> , <i>Gigaspora</i> , <i>Acaulospora</i> sp. + <i>Bacillus megaterium</i> , <i>Azospirillum amazonense</i> , <i>Azotobacter</i> sp.	<i>Curcuma longa</i> L.	Increased flavonoids, phenolic and curcumin content	Dutta and Neog (2016)
Fungal endophyte + PGPR(s)			
<i>P. indica</i> + <i>A. chroococcum</i>	<i>Stevia rebaudiana</i>	Enhanced steviol glycoside (stevioside and rebaudioside-A) content	Kilam et al. (2015)
<i>P. indica</i> + <i>A. chroococcum</i>	<i>Artemisia annua</i>	Enhanced artemisinin content	Arora et al. (2016)

16.4 Effect of Microbial Symbiosis on Plant Bioactive Compounds

The increase in the concentration of plant bioactive compounds upon symbiotic association of microorganisms has in general been considered a defence response of the host plant. However, it is not clear how microbial association causes changes to the phytochemicals in the plant (Toussaint 2007). The improved quantity and quality of bioactive compounds can be attributed to changes in the plant nutritional and non-nutritional pathways.

Phosphorus is an important constituent of the intermediates of secondary metabolite biosynthetic pathways. It is also an important constituent of nucleic acids and bio membranes (Marschner 2011) and an important source for essential oil synthesis by plants (Lichtenthaler 2009). Phosphorus fertilization has shown to increase the secondary metabolite content in both mycorrhizal and non-mycorrhizal plants, indicating its role in secondary metabolite accumulation (Abu-Zeyad et al. 1999). Bacterial and mycorrhizal fungi symbioses have shown to increase the phosphorus acquisition efficiency of plants by releasing phosphatase enzymes or organic acids that make phosphorus available in the organic form (Malla et al. 2004; Smith and

Read 2008). However, the microbes in the rhizosphere have different capacities to solubilize phosphorous and therefore could differently affect its availability to plants. Mycorrhizal inoculation at higher levels of phosphorus in medicinal plant *Anadenanthera colubrina* showed increased concentration of secondary metabolites (Pedone-Bonfim et al. 2013). Study in *Salvia officinalis* has shown that phosphorus fertilization alone has a better enhancement effect on secondary metabolite content as compared to AMF-inoculated plants (Nell et al. 2009). Another study in *Ocimum basilicum* showed higher production of antioxidants, rosmarinic and caffeic acids in mycorrhizal plants, as compared to non-mycorrhizal plants with higher phosphorus amendments (Toussaint et al. 2007). Plant-associated symbionts are also known to maintain the availability of other plant nutrients such as nitrogen, potassium, magnesium and microelements (Maheshwari et al. 2012). Sharma et al. (2014) reported significant increase in phytochemical content and radical-scavenging activity upon inoculation of *A. vera* plantlets with *P. indica*. In another study, Selvaraj and Sumithra (2011) also showed an increase in root phosphorus, potassium, zinc, copper and iron contents after inoculation with consortium of AM fungi and PGPR(s). Similar results were observed by Singh et al. (2013) where macronutrients increased upon inoculation with *Pseudomonas monteilii* and *G. fasciculatum*.

Medicinal plants are widely affected by abiotic stress which has a direct impact on plant growth and production of bio active compounds. Microbial mediated alleviation of abiotic stress helps in reducing this negative effect on plants. Medicinal plant, *Hyoscyamus niger* which is a source of important tropane alkaloids, showed reduced plant growth and development under water stress. To alleviate the water stress, *Pseudomonas putida* and *P. fluorescens* were inoculated, which led to increase in plant growth and alkaloid content in *H. niger* (Ghorbanpour et al. 2013). Similar study was also undertaken in *Pelargonium* sp. where salinity stress decreased the plant growth and essential oil content in plants. Combined effects of AMF, *G. intraradices*, and PGPR, *P. fluorescens*, were studied which showed increased plant growth, nutrient uptake and essential oil contents (Prasad et al. 2012).

16.5 Conclusion and Future Prospects

This chapter highlights the use of microbial symbionts to improve the quantity and quality of bioactive compounds in medicinal plants. The plant-microbe symbiotic interaction provides a promising strategy for the better establishment of plants and also for enhancement of various phytochemicals present in the plant. A wide variety of fungi including AM fungi, root endophytes, and bacteria is recognized in the rhizosphere that has significant effect on the bioactive constituents of medicinal plants. However, selection of an efficient bacteria/or fungi for a specific medicinal plant is mandatory for the best response to obtain higher quantity and quality of their products. Therefore, further research is recommended to better understand the

function and diversity of interaction between symbiotic microbes and medicinal plants and also to elucidate the involved mechanisms.

Acknowledgements Authors are grateful to DBT for partial financial assistance and DST for providing confocal microscope.

References

- Abu-Zeyad R, Khan AG, Khoo C (1999) Occurrence of Arbuscular Mycorrhiza in *Castanospermum australe* A. Cunn. and C. Fraser and effects on growth and production of castanospermine. *Mycorrhiza* 9:111–117
- Ahlawat S, Saxena P, Ali A, Abdin M Z (2016) *Piriformospora indica* elicitation of withaferin A biosynthesis and biomass accumulation in cell suspension cultures of *Withania somnifera*. *Symbiosis* 1–10. doi:10.1007/s13199-015-0364-9
- Alam M, Khaliq A, Sattar A, Shukla RS, Anwar M, Dharni S (2011) Synergistic effect of Arbuscular Mycorrhizal fungi and *Bacillus subtilis* on the biomass and essential oil yield of rose-scented geranium (*Pelargonium graveolens*). *Arch Agron Soil Sci* 57:889–898
- Algar E, Gutierrez-Mañero FJ, Bonilla A, Lucas JA, Radzki W, Ramos-Solano B (2012) *Pseudomonas fluorescens* N21. 4 metabolites enhance secondary metabolism isoflavones in soybean (*Glycine max*) calli cultures. *J Agric Food Chem* 60:11080–11087
- Araim G, Saleem A, Arnason JT, Charest C (2009) Root colonization by an Arbuscular Mycorrhizal (AM) fungus increases growth and secondary metabolism of purple coneflower, *Echinacea purpurea* (L.) Moench. *J Agric Food Chem* 57:2255–2258
- Arora M, Saxena P, Choudhary DK, Abdin MZ, Varma A (2016) Dual symbiosis between *Piriformospora indica* and *Azotobacter chroococcum* enhances the artemisinin content in *Artemisia annua* L. *World J Microbiol Biotechnol* 32:1–10
- Arpana J, Bagyaraj DJ, Prakasa Rao EVS, Parameswaran TN, Abdul Rahiman BA (2008) Symbiotic response of patchouli [*Pogostemon cablin* (Blanco) Benth.] to different Arbuscular Mycorrhizal fungi. *Adv Environ Biol* 2:20–24
- Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Sci* 166:3–16
- Bagde US, Prasad R, Varma A (2010) Interaction of *Piriformospora indica* with medicinal plants and of economic importance. *Afr J Biotechnol* 9:9214–9226
- Bagde US, Prasad R, Varma A (2014) Impact of culture filtrate of *Piriformospora indica* on biomass and biosynthesis of active ingredient aristolochic acid in *Aristolochia elegans* Mart. *Int J Biol* 1:29–37
- Bahadori F, Ashorabadi ES, Mirza M, Matinizade M, Abdosi V (2013) Improved growth, essential oil yield and quality in *Thymus daenensis* Celak on mycorrhizal and plant growth promoting rhizobacteria inoculation. *Int J Agron Plant Prod* 4:3384–3391
- Bajaj R, Agarwal A, Rajpal K, Asthana S, Prasad R, Kharkwal AC, Kumar R, Sherameti I, Oelmüller R, Varma A (2014) Co-cultivation of *Curcuma longa* with *Piriformospora indica* enhances the yield and active ingredients. *Am J Curr Microbiol* 2:6–17
- Baldi A, Jain A, Gupta N, Srivastava AK, Bisaria VS (2008) Co-culture of Arbuscular Mycorrhiza-like fungi (*Piriformospora indica* and *Sebacinia vermifera*) with plant cells of *Linum album* for enhanced production of podophyllotoxins: a first report. *Biotechnol Lett* 30:1671–1677
- Banchio E, Bogino PC, Zygadlo J, Giordano W (2008) Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana* L. *Biochem Syst Ecol* 36:766–771

- Banchio E, Xie X, Zhang H, Pare PW (2009) Soil bacteria elevate essential oil accumulation and emissions in sweet basil. *J Agric Food Chem* 57:653–657
- Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. *J Exp Bot* 56:1761–1778
- Basiewicz M, Weiß M, Kogel KH, Langen G, Zorn H, Zuccaro A (2012) Molecular and phenotypic characterization of *Sebacina vermifera* strains associated with orchids, and the description of *Piriformospora williamsii* sp. nov. *Fungal Biol* 116:204–213
- Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb Cell Factories* 13:66–76
- Bharti N, Yadav D, Barnawal D, Maji D, Kalra A (2013) *Exiguobacterium oxidotolerans*, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in *Bacopa monnieri* (L.) Pennell under primary and secondary salt stress. *World J Microbiol Biotechnol* 29:379–387
- Bhuyan SK, Bandyopadhyay P, Kumar P, Mishra DK, Prasad R, Kumari A, Upadhyaya KC, Varma A, Yadava PK (2015) Interaction of *Piriformospora indica* with *Azotobacter chroococcum*. *Sci Rep* 5:13911. doi:10.1038/srep13911
- Bothe H, Turnau K, Regvar M (2010) The potential role of Arbuscular Mycorrhizal fungi in protecting endangered plants and habitats. *Mycorrhiza* 20:445–457
- Bottcher H (1965) Miracle drugs. Zora, Zagreb, pp 23–139
- Bucio JL, Campos-Cuevas JC, Hernandez-Calderon E, Valasquez-Bacerra C, Farias-Rodriguez R, Macias-Rodriguez LI, Valencia-Cantero E (2007) *Bacillus megaterium* rhizobacteria promote growth and alter root system architecture through an auxin and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. *Mol Plant-Microbe Interact* 20:207–217
- Carroll G (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecol* pp:2–9
- Chadha N, Mishra M, Prasad R, Varma A (2014) Root endophytic fungi: Research update. *J Biol Life Sci USA* 5:135–158
- Chaudhary V, Kapoor R, Bhatnagar AK (2008) Effectiveness of two Arbuscular Mycorrhizal fungi on concentrations of essential oil and artemisinin in three accessions of *Artemisia annua* L. *Appl Soil Ecol* 40:174–181
- Clark RB, Zeto SK (2000) Mineral acquisition by Arbuscular Mycorrhizal plants. *J Plant Nutr* 23:867–902
- Copetta A, Lingua G, Berta G (2006) Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. *Mycorrhiza* 16:485–494
- Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). *Biochem Mol Biol Plant* 24:1250–1319
- Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Varma A (2012) The root endophyte fungus *Piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. *Plant Signal Behav* 7:103–112
- Das A, Prasad R, Srivastava RB, Deshmukh S, Rai MK, Varma A (2013) Co-cultivation of plants with medicinal plants: case studies. In: Varma A, Kost G, Oelmüller R (eds) *Piriformospora indica*: sebacinales and their biotechnological applications. Springer, Berlin, pp 149–171
- Dastborhan S, Zehtab-Salmasi S, Nasrollahzadeh S, Tavassoli AR (2011) Effect of plant growth-promoting rhizobacteria and nitrogen fertilizer on yield and essential oil of german chamomile (*Matricaria chamomilla* L.). *International Symposium on Medicinal and Aromatic Plants IMAPS2010 and History of Mayan Ethnopharmacology IMAPS2011* 964, pp 121–128
- De la Rosa-Mera CJ, Ferrera-Cerrato R, Alarcón A, De Jesús Sánchez-Colín M, Muñoz-Muñoz OD (2011) Arbuscular Mycorrhizal fungi and potassium bicarbonate enhance the foliar content of the vinblastine alkaloid in *Catharanthus roseus*. *Plant Soil* 349:367–376

- Del Rosario CL, Santoro MV, Nieves F, Giordano W, Banchio E (2013) Increase of secondary metabolite content in marigold by inoculation with plant growth-promoting rhizobacteria. *Appl Soil Ecol* 70:16–22
- Del Rosario CL, Santoro MV, Reinoso H, Travaglia C, Giordano W, Banchio E (2015) Anatomical, morphological, and phytochemical effects of inoculation with plant growth-promoting rhizobacteria on peppermint (*Mentha piperita*). *J Chem Ecol* 41:149–158
- Dutta SC, Neog B (2016) Accumulation of secondary metabolites in response to antioxidant activity of turmeric rhizomes co-inoculated with native Arbuscular Mycorrhizal fungi and plant growth promoting rhizobacteria. *Sci Hortic* 204:179–184
- Fan JH, Yang GT, Mu LQ, Zhou JH (2006) Effect of AM fungi on the content of berberine, jatrorrhizine and palmatine of *Phellodendron amurense* seedlings. *Protect Forest Sci Technol* 5:24–26
- Geneva MP, Stancheva IV, Boychinova MM, Mincheva NH, Yonova PA (2010) Effects of foliar fertilization and arbuscular mycorrhizal colonization on *Salvia officinalis* L. growth, antioxidant capacity, and essential oil composition. *J Sci Food Agric* 90:696–702
- Ghorbanpour M, Majnoun HN, Rezazadesh SA, Omid M, Khavazi K, Hatami M (2011) Variations of root and shoot tropane alkaloids production of *Hyoscyamus niger* under two rhizobacteria strains inoculation and water deficit stress. *J Med Plants* 10:160–170
- Ghorbanpour M, Hatami M, Khavazi K (2013) Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of *Hyoscyamus niger* under water deficit stress. *Turk J Biol* 37:350–360
- Ghorbanpour M, Hosseini N, Khodae Motlagh M, Solgi M (2014) The effects of inoculation with pseudomonads rhizobacteria on growth. Quantity and quality of essential oils in sage (*Salvia officinalis* L). *Plant J Med Plants* 4:89–100
- Gianinazzi S, Gollotte A, Binet MN, Van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* 20:519–530
- Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. *Front Microbiol* 7:332. doi:10.3389/fmicb.2016.00332
- Glesinger L (1954) *Medicine through centuries*. Zora, Zagreb, pp 21–38
- Gorelick J, Bernstein N (2014) Elicitation: an underutilized tool for the development of medicinal plants as a source for therapeutic secondary metabolites. *Adv Agron* 124:201–230
- Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food Agric* 2:1127500
- Großkinsky DK, Van der Graaff E, Roitsch T (2016) Regulation of abiotic and biotic stress responses by plant hormones. *Plant Pathogen Resist Biotechnol* 131. doi:10.1002/9781118867716.ch7
- Gu R, Wang Y, Long B, Kennelly E, Wu S, Liu B, Long C (2014) Prospecting for bioactive constituents from traditional medicinal plants through ethnobotanical approaches. *Biol Pharm Bull* 37:903–915
- Guenther E (2013) *The essential oils, History-origin in plants-production-analysis*, vol 1. Read Books, New York
- Guo Q, Cheng L, Liu Z (2010) Study on influence of arbuscular mycorrhizal fungi *Pinellia ternata* yield and chemical composition. *Zhongguo Zhong yao za zhi Zhongguo zhongyao zazhi. J Chinese Materia Medica* 35:333–338
- Gupta ML, Prasad A, Ram M, Kumar S (2002) Effect of the Vesicular–Arbuscular Mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol mint (*Mentha arvensis*) under field conditions. *Bioresour Technol* 81:77–79
- Harman GE (2011) Multifunctional fungal plant symbiont: new tools to enhance plant growth and productivity. *New Phytol* 189:647–649
- Harrison MJ (1999) Molecular and cellular aspects of the Arbuscular mycorrhizal symbiosis. *Annu Rev Plant Physiol Plant Mol Biol* 50:361–389

- Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* 13:572–584
- Hemashenpagam N, Selvaraj T (2011) Effect of Arbuscular Mycorrhizal (AM) fungus and plant growth promoting rhizo-microorganisms (PGPR's) on medicinal plant *Solanum viarum* seedlings. *J Environ Biol* 32:579. doi:10.3389/fpls.2013.00356
- Hermosa R, Viterbo A, Chet I, Monte E (2012) Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology* 158:17–25
- Honggang GW (1989) Effects of VA mycorrhizal fungi on growth, nutrient uptake and effective compounds in chinese medicinal herb *Datura stramonium* L. *Sci Agric Sin* 5:008
http://www.centerfortraditionalmedicine.org/uploads/2/3/7/5/23750643/medicinal_plants_in_china.pdf. Accessed on 27 Apr 2016
- Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R, Panneerselvam R (2007) *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. *Colloids Surf B Biointerfaces* 60:7–11
- Jasim B, Geethu PR, Mathew J, Radhakrishnan EK (2015) Effect of endophytic *Bacillus* sp. from selected medicinal plants on growth promotion and diosgenin production in *Trigonella foenum-graecum*. *Plant Cell Tissue Organ Cult* 122:565–572
- Johnson JM, Alex T, Oelmüller R (2014) *Piriformospora indica*: the versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants. *J Trop Agric* 52:103–122
- Jurkiewicz A, Ryszka P, Anielska T, Waligórski P, Białońska D, Góralska K, Turnau K (2010) Optimization of culture conditions of *Arnica montana* L.: effects of mycorrhizal fungi and competing plants. *Mycorrhiza* 20:293–306
- Kapoor R, Giri B, Mukerji KG (2002) *Glomus macrocarpum*: a potential bioinoculant to improve essential oil quality and concentration in Dill (*Anethum graveolens* L.) and Carum (*Trachyspermum ammi* (Linn.) Sprague). *World J Microbiol Biotechnol* 18:459–463
- Kapoor R, Giri B, Mukerji KG (2004) Improved growth and essential oil yield and quality in *Foeniculum vulgare* mill on mycorrhizal inoculation supplemented with P-fertilizer. *Bioresour Technol* 93:307–311
- Kapoor R, Chaudhary V, Bhatnagar AK (2007) Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* 17:581–587
- Karagiannidis N, Thomidis T, Lazari D, Panou-Filothou E, Karagiannidou C (2011) Effect of three Greek arbuscular mycorrhizal fungi in improving the growth, nutrient concentration, and production of essential oils of oregano and mint plants. *Sci Hortic* 129:329–334
- Karthikeyan B, Jaleel CA, Azooz MM (2009) Individual and combined effects of *Azospirillum brasilense* and *Pseudomonas fluorescens* on biomass yield and ajmalicine production in *Catharanthus roseus*. *Acad J Plant Sci* 2:69–73
- Karthikeyan B, Joe MM, Jaleel CA, Deiveekasundaram M (2010) Effect of root inoculation with plant growth promoting rhizobacteria (PGPR) on plant growth, alkaloid content and nutrient control of *Catharanthus roseus* (L.) G. Don. *Natura Croatica* 19:205–212
- Khaosaad T, Vierheilig H, Nell M, Zitterl-Eglseer K, Novak J (2006) Arbuscular mycorrhiza alter the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae). *Mycorrhiza* 16: 443–446
- Kilam D, Saifi M, Abdin MZ, Agnihotri A, Varma A (2015) Combined effects of *Piriformospora indica* and *Azotobacter chroococcum* enhance plant growth, antioxidant potential and steviol glycoside content in *Stevia rebaudiana*. *Symbiosis* 66:149–156
- Knapp DG, Kovács GM, Zajta E, Groenewald JZ, Crous PW (2015) Dark septate endophytic pleosporalean genera from semiarid areas. *Persoonia-Mol Phylogeny Evol Fungi* 35:87–100
- Köberl M, Schmidt R, Ramadan EM, Bauer R, Berg G (2015) The microbiome of medicinal plants: diversity and importance for plant growth, quality, and health. The plant microbiome and its importance for plant and human health. doi:10.1128/MMBR.00050-14
- Kogel KH, Franken P, Hüchelhoven R (2006) Endophyte or parasite-what decides? *Curr Opin Plant Biol* 9:358–363

- Kohler J, Caravaca F, Carrasco L, Roldan A (2007) Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of *Lactuca sativa*. *Appl Soil Ecol* 35:480–487
- Lee J, Scagel CF (2009) Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. *Food Chem* 115:650–656
- Lermen C, Morelli F, Gazim ZC, Da Silva AP, Gonçalves JE, Dragunski DC, Alberton O (2015) Essential oil content and chemical composition of *Cymbopogon citratus* inoculated with arbuscular mycorrhizal fungi under different levels of lead. *Ind Crop Prod* 76:734–738
- Lichtenthaler HK (2009) Biosynthesis and accumulation of isoprenoid carotenoids and chlorophylls and emission of isoprene by leaf chloroplasts. *Bull Geogr Nat Acad Sci* 3:81–94
- Liu J, Wu L, Wei S, Xiao X, Su C, Jiang P, Yu Z (2007) Effects of arbuscular mycorrhizal fungi on the growth, nutrient uptake and glycyrrhizin production of licorice (*Glycyrrhiza uralensis* Fisch.). *J Plant Growth Regul* 52:29–39
- Lum MR, Hirsch AM (2003) Roots and their symbiotic microbes: strategies to obtain nitrogen and phosphorus in a nutrient-limiting environment. *J Plant Growth Regul* 21:368–382
- Maheshwari DK, Dubey RC, Aeron A, Kumar B, Kumar S, Tewari S, Arora NK (2012) Integrated approach for disease management and growth enhancement of *Sesamum indicum* L. utilizing *Azotobacter chroococcum* TRA2 and chemical fertilizer. *World J Microbiol Biotechnol* 28:3015–3024
- Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmüller R, Varma A (2004) Phosphorus solubilizing symbiotic fungus: *Piriformospora indica*. *Endocytobiosis Cell Res* 15:579–600
- Mandal S, Evelin H, Giri B, Singh VP, Kapoor R (2013) Arbuscular mycorrhiza enhances the production of stevioside and rebaudioside-A in *Stevia rebaudiana* via nutritional and non-nutritional mechanisms. *Appl Soil Ecol* 72:187–194
- Mandal S, Upadhyay S, Wajid S, Ram M, Jain DC, Singh VP, Kapoor R (2015) Arbuscular mycorrhiza increase artemisinin accumulation in *Artemisia annua* by higher expression of key biosynthesis genes via enhanced jasmonic acid levels. *Mycorrhiza* 25:345–357
- Marschner H (2011) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, Cambridge, 651 pp. Hardcover ISBN: 9780123849052
- Martinez MJA, Lazaro RM, Del Olmo LMB, Benito PB (2008) Anti-infectious activity in the anthemideae tribe. *Stud Nat Prod Chem* 35:445–516
- Mayerhofer MS, Kernaghan G, Harper KA (2013) The effects of fungal root endophytes on plant growth: a meta-analysis. *Mycorrhiza* 23:119–128
- McKersie DB (1996) Oxidative stress. <http://www.agronomy.psu.edu/Courses/AGRO518/Oxygen.htm>. Accessed 25 Oct 2000
- Meena KK, Mesapogu S, Kumar M, Yandigeri MS, Singh G, Saxena AK (2010) Co-inoculation of the endophytic fungus *Piriformospora indica* with the phosphate-solubilising bacterium *Pseudomonas striata* affects population dynamics and plant growth in chickpea. *Biol Fertil Soils* 46:169–174
- Meng JJ, He XL (2011) Effects of AM fungi on growth and nutritional contents of *Salvia miltiorrhiza* Bge. under drought stress. *J Agr Univ Hebei* 34:51–61
- Morone-Fortunato I, Avato P (2008) Plant development and synthesis of essential oils in micro-propagated and mycorrhiza inoculated plants of *Origanum vulgare* L. ssp. *hirtum* (Link) Letswaart. *Plant Cell Tissue Organ Cult* 93:139–149
- Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora indica* interaction under stress condition. *Front Plant Sci* 7:1574. doi:10.3389/fpls.2016.01574
- Nautiyal CS, Chauhan PS, Das Gupta SM, Seem K, Varma A, Staddon WJ (2010) Tripartite interactions among *Paenibacillus lentimorbus* NRRL B-30488, *Piriformospora indica* DSM 11827, and *Cicer arietinum* L. *World J Microbiol Biotechnol* 26:1393–1399
- Nell M, Voetsch M, Vierheilg H, Steinkellner S, Zitterl-Eglseer K, Franz C, Novak J (2009) Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.) *J Sci Food Agric* 89:1090–1096

- Oliveira MS, Campos MA, Silva FS (2015) Arbuscular mycorrhizal fungi and vermi compost to maximize the production of foliar biomolecules in *Passiflora alata* Curtis seedlings. *J Sci Food Agric* 95:522–528
- Pandey MM, Rastogi S, Rawat AKS (2013) Indian traditional ayurvedic system of medicine and nutritional supplementation. *Evid Based Complement Alternat Med* 2013:1–12. doi:10.1155/2013/376327
- Paszkowski U (2006) Mutualism and parasitism: the yin and yang of plant symbioses. *Curr Opin Plant Biol* 9:364–370
- Pedone-Bonfim MV, Lins MA, Coelho IR, Santana AS, Silva FS, Maia LC (2013) Mycorrhizal technology and phosphorus in the production of primary and secondary metabolites in cebil (*Anadenanthera colubrina* (Vell.) Brenan) seedlings. *J Sci Food Agric* 93:1479–1484
- Prasad A, Kumar S, Pandey A, Chand S (2012) Microbial and chemical sources of phosphorus supply modulate the yield and chemical composition of essential oil of rose-scented geranium (*Pelargonium* species) in sodic soils. *Biol Fertil Soils* 48:117–122
- Prasad R, Kamal S, Sharma PK, Oelmüller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. *J Basic Microbiol* 53:1016–1024
- Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) *Plant growth-promoting rhizobacteria and medicinal plants*. Springer, Cham, pp 247–260
- Rajasekar S, Elango R (2011) Effect of microbial consortium on plant growth and improvement of alkaloid content in *Withania somnifera* (Ashwagandha). *Curr Bot* 2:27–30
- Refish NMR, Talib AJ, Jian-Wei G, Fu C, Yu L (2016) Promoting role of *Bacillus Subtilis* BS87 on the growth and content of some natural products in the medicinal plants *Anoectochilus Roxburghii* and *A. Formosanus*. *Adv Life Sci* 6:31–38
- Rojas-Andrade R, Cerda-García-Rojas C, Frías-Hernández J, Dendooven L, Olalde-Portugal V, Ramos-Valdivia A (2003) Changes in the concentration of trigonelline in a semi-arid leguminous plant (*Prosopis laevigata*) induced by an arbuscular mycorrhizal fungus during the pre-symbiotic phase. *Mycorrhiza* 13:49–52
- Sailo GL, Bagyaraj DJ (2005) Influence of different AM-fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*. *Mycol Res* 109:795–798
- Santoro MV, Zygadlo J, Giordano W, Banchio E (2011) Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). *Plant Physiol Biochem* 49:1177–1182
- Satheesan J, Narayanan AK, Sakunthala M (2012) Induction of root colonization by *Piriformospora indica* leads to enhanced asiaticoside production in *Centella asiatica*. *Mycorrhiza* 22:195–202
- Schulz B (2006) Mutualistic interactions with fungal root endophytes. In: Schulz B, Boyle C, Sieber T (eds) *Microbial root endophytes*. Springer, Berlin, pp 261–279
- Schulz B, Boyle C (2005) The endophytic continuum. *Mycol Res* 109:661–686
- Selvaraj T, Sumithra P (2011) Effect of *Glomus aggregatum* and plant growth promoting rhizomicroorganisms on growth, nutrition and content of secondary metabolites in *Glycyrrhiza glabra* L. *Indian J Appl Pure Biol* 26:283–290
- Sharma D, Kapoor R, Bhatnagar AK (2007) Arbuscular mycorrhizal (AM) technology for the conservation of *Curculigo orchioides* Gaertn.: an endangered medicinal herb. *World J Microbiol Biotechnol* 24:395–400
- Sharma G, Agrawal V (2013) Marked enhancement in the artemisinin content and biomass productivity in *Artemisia annua* L. shoots co-cultivated with *Piriformospora indica*. *World J Microbiol Biotechnol* 29:1133–1138
- Sharma P, Kharkwal AC, Abdin MZ, Varma A (2014) *Piriformospora indica* improves micro propagation, growth and phytochemical content of *Aloe vera* L. *Plants. Symbiosis* 64:11–23

- Sieniawska E, Baj T, Dudka J, Gieroba R, Swiatek L, Rajtar B, Polz-Dacewicz M (2013) Cytotoxicity, antioxidant activity and an effect on CYP3A4 and CYP2D6 of *Mutellina purpurea* L. extracts. *Food Chem Toxicol* 52:188–192
- Singh A, Sharma J, Rexer KH, Varma A (2000) Plant productivity determinants beyond minerals, water and light: *Piriformospora indica* – a revolutionary plant growth promoting fungus. *Curr Sci* 79:1548–1554
- Singh AN, Singh AR, Kumari M, Rai MK, Varma A (2003) Biotechnology importance of *Piriformospora indica* – a novel symbiotic mycorrhiza-like fungus: an overview. *Indica J Biotechnol* 2:65–75
- Singh R, Soni SK, Kalra A (2013) Synergy between *Glomus fasciculatum* and a beneficial *Pseudomonas* in reducing root diseases and improving yield and forskolin content in *Coleus forskohlii* Briq. under organic field conditions. *Mycorrhiza* 23:35–44
- Smith SE, Read DJ (2008) *Mycorrhizal symbiosis*. Academic, London, p 800
- Teng HR, He XL (2005) Effects of different AM fungi and N levels on the flavonoid content of *Bupleurum scorzonrifolium* Wild. *J Shanxi Agric Sci* 4:53–54
- Toro M, Azcon R, Barea J (1997) Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability ((sup32) P) and nutrient cycling. *Appl Environ Microbiol* 63:4408–4412
- Toussaint JP (2007) Investigating physiological changes in the aerial parts of AM plants: what do we know and where should we be heading? *Mycorrhiza* 17:349–353
- Toussaint JP, Smith FA, Smith SE (2007) Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. *Mycorrhiza* 17:291–297
- Tucakov J (1971) Healing with plants–phytotherapy. Culture, Beograd, pp 180–190
- Turnau K, Haselwandter K (2002) Arbuscular mycorrhizal fungi an essential component of soil microflora in ecosystem restoration. In: Gianinazzi S, Schuepp H, Barea JM, Haselwandter K (eds) *Mycorrhizal technology in agriculture from genes to mycorrhiza application*. Birkhauser Verlag, Basel, pp 137–149
- Urcoviche RC, Gazim ZC, Dragunski DC, Barcellos FG, Alberton O (2015) Plant growth and essential oil content of *Mentha crispera* inoculated with arbuscular mycorrhizal fungi under different levels of phosphorus. *Ind Crop Prod* 67:103–107
- Vafadar F, Amooaghaie R, Otroshy M (2014) Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *J Plant Interact* 9:128–136
- Varma A, Verma S, Sudha Sahay N, Buttehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant growth promoting root endophyte. *Appl Environ Microbiol* 65:2741–2744
- Varma A, Singh A, Sudha Sahay NS, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Hurek T, Blechert O, Rexer KH, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2000) *Mycota*, vol IX. Springer, Berlin, Chapter 8, pp 225–253
- Varma A, Singh A, Sudha Sahay N, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T, Blechert O, Rexer KH, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica*: an axenically culturable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) *Fungal associations, The mycota*, vol IX. Springer, Berlin, pp 123–150
- Varma A, Sherameti I, Tripathi S, Prasad R et al (2012) The symbiotic fungus *Piriformospora indica*: review. In: Hock B (ed) *Fungal associations, The mycota*, vol IX, 2nd edn. Springer, Berlin, pp 231–254
- Wei G, Wang H (1991) Effect of vesicular-arbuscular mycorrhizal fungi on growth, nutrient uptake and synthesis of volatile oil in *Schizonepeta tenuifolia* briq. *Zhongguo Zhong yao za zhi*= *Zhongguo zhongyao zazhi*. *Chinese J Materia Medica* 16:139–142
- Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *J Biol Chem* 285:26532–26544

- Zhao JL, He XL (2011) Effects of AM fungi on drought resistance and content of chemical components in *Angelica dahurica*. *Acta Botan Boreali-Occiden Sin* 20:184–189
- Zhao X, Wang BW, Yan XF (2006) Effect of arbuscular mycorrhiza on camptothecin content in *Camptotheca acuminata* seedlings. *Acta Ecol Sin* 26:1057–1062
- Zhao JL, Deng HY, He XL (2009) Effects of AM fungi on the quality of trueborn *Angelica dahurica* from Hebei province. *Acta Agric Bor Sin* 24:299–302
- Zhao JL, Zhou LG, JY W (2010) Promotion of *Salvia miltiorrhiza* hairy root growth and tanshinone production by polysaccharide–protein fractions of plant growth-promoting rhizobacterium *Bacillus cereus*. *Process Biochem* 45:1517–1522
- Zhong JH, Fan JH (2007) Effects of AM fungi on the berberine content in *Phellodendron chinense* seedlings [J]. *Nat Hortic* 12:013
- Zhu ZB, Fan JY, Guo QS, Liu ZY, Zhu GS (2015) The growth and medicinal quality of *Epi-medium wushanense* are improved by an isolate of dark septate fungus. *Pharma Biol* 53: 1344–1351
- Ziegler J, Facchini PJ (2008) Alkaloid biosynthesis: metabolism and trafficking. *Annu Rev Plant Biol* 59:735–769
- Zitterl-Eglseer K, Nell M, Lamien-Meda A, Steinkellner S, Wawrosch C, Kopp B, Novak J (2015) Effects of root colonization by symbiotic arbuscular mycorrhizal fungi on the yield of pharmacologically active compounds in *Angelica archangelica* L. *Acta Physiol Plant* 37:1–11
- Zubek S, Stojakowska A, Anielska T, Turnau K (2010) Arbuscular mycorrhizal fungi alter thymol derivative contents of *Inula ensifolia* L. *Mycorrhiza* 20:497–504
- Zubek S, Mielcarek S, Turnau K (2012) Hypericin and pseudohypericin concentrations of a valuable medicinal plant *Hypericum perforatum* L. are enhanced by arbuscular mycorrhizal fungi. *Mycorrhiza* 22:149–156
- Zubek S, Rola K, Szewczyk A, Majewska ML, Turnau K (2015) Enhanced concentrations of elements and secondary metabolites in *Viola tricolor* L. induced by arbuscular mycorrhizal fungi. *Plant Soil* 390:129–142

Chapter 17

Cultivation of *Piriformospora indica* with Nanomaterial in Bioreactor

Uma and Ajit Varma

Abstract *Piriformospora indica* is an axenically cultivable root endophytic fungus which exerts plant growth-promoting effects on its host plants. To enable commercial production of its spores, *Piriformospora indica* was cultivated in association of nanostructured materials “zinc oxide” in a 7 L batch bioreactor called “nanoembedded fungus” a novel nano-tool such that they result in maximum biomass during growth phase and in maximum spore yield during subsequent sporulation phase. An enhancement in overall biomass productivity of about 50% when *P. indica* was grown with zinc oxide nanorods and also the maximum spore yield (9.25×10^9 spores/mL) was achieved in comparison to the cultivation of *P. indica* alone (without inclusion of nanomaterials) in bioreactor. The high spore yield obtained when cultivated with zinc nanomaterials in the chapter seems to be economical for commercial production of *P. indica*.

17.1 Introduction

Nanotechnology is an enabling technology dealing with nanometer-sized particles. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties (Prasad et al. 2016). Now they have entered a commercial exploration period in the area of biotechnology, leading to the development of a new field of science nanobiotechnology (Murray et al. 2000). Understanding of biological processes at the nanoscale level is a strong driving force behind the development of nanobiotechnology (Whitesides 2003) as it is well known; living organisms are built of cells that are typically 10 μ m across. However, the biomolecules are much smaller and are in the submicron size domain. Even smaller are the proteins with a typical size of just 5 nm, comparable with the smallest man-made nanoparticles. This simple size comparison gives an idea of using nanoparticles as nanoprobe that would allow us to spy at the cellular machinery without too much interference (Taton 2002).

Uma • A. Varma (✉)

Amity Institute of Microbial Technology, Amity University, Sector-125, Noida 201303, Uttar Pradesh, India

e-mail: ajitvarma@amity.edu

In order to enhance the utilization of nanomaterials in biological systems, it is important to understand the influence they have on the cellular health and function (Suman et al. 2010). Nanomaterials present a research challenge as little is known about how they behave in relation to microorganisms, particularly at the cellular level. Most of the nanomaterials reported earlier have been demonstrated to be efficient antimicrobial agents (Raffi et al. 2008; Aziz et al. 2015, 2016). There are only a few or no reports on the growth-promoting role of the nanomaterials, especially with respect to microbes (Suman et al. 2010). It has been reported that nanoparticles possess more surface area than microparticles, thus improving the physical and chemical characteristics of a particle, which may also influence the biological property of the material. Zinc oxide (ZnO), which can exhibit a wide variety of nanostructures, possesses unique semiconducting and optical properties. One of the most important features of ZnO nanomaterials is low toxicity and biodegradability. Zn²⁺ is an indispensable trace element for adults (~10 mg of Zn²⁺ per day is recommended), and it is involved in various aspects of metabolism. Chemically, the surface of ZnO is rich in –OH groups, which can be readily functionalized by various surface decorating molecules. ZnO nanomaterials are important for its biomedical applications, such as biomedical imaging (which includes fluorescence, magnetic resonance, positron emission tomography, as well as dual-modality imaging), drug delivery, gene delivery, biocompatible/biodegradable, biosensing, photocatalysis, and antibacterial applications (Bhuyan et al. 2015a, b; Hatamie et al. 2015).

In the present chapter, the interaction of nanomaterials with the fungal endophyte *Piriformospora indica* and their effect on the growth processes at different stages of development have been studied (Suman et al. 2010). The fungus was chosen as a representative model to observe the effect of nanoparticles on growth enhancement. This property has already been patent granted (Varma et al. 2015, Patent Number: 267958).

17.2 Methods

17.2.1 Synthesis of Zinc Oxide Nanorods

Zinc oxide nanorods were synthesized by using mechanically assisted thermal decomposition method described by Bhuyan et al. (2015a) and characterized by XRD, UV-Vis, and scanning electron microscopy.

17.2.2 Microorganism, Culture Maintenance, and Inoculum Preparation

Usually the stock culture is maintained on slants containing Hill and Kaefer medium (Prasad et al. 2005, 2013) supplemented with 15 g/L agar. Inoculate the slants, incubate at 30 °C for 8 days, and then store at 4 °C. For the preparation of inoculum, grow *P. indica* on Kaefer medium in a petri dish (Hill and Kaefer 2001).

During the interaction between nanomaterials and *P. indica*, a zinc oxide (ZnO) nanomaterial was used. ZnO was mixed (50 mg/100 mL) in Hill and Kaefer broth with 2% glucose, in separate Erlenmeyer flasks with initial pH adjusted to 6.4. Usually 4–5 fully grown fungal agar discs (4 mm in diameter) were inoculated into each 500 mL Erlenmeyer flask containing 100 mL of Hill and Kaefer broth medium. Flasks were incubated for 7–10 days at 28 + 2 °C with constant shaking at 100 rotations/min on a rotary shaker.

17.3 Production of Fungal Culture with Nanomaterial in Bioreactor

Bioreactor provides optimized environmental and nutritional conditions for the large-scale production of microbial cultures. The constant administration of conditions at variable stages in bioreactor enables a more efficient scale-up of microbial cultures. The submerged conditions enhance the uptake of nutrients resulting in stimulation of the biochemical processes. Batch culture comprises of a closed system which encompasses an initial restricted availability of nutrient. The batch fermentation is employed for the production of nanoembedded fungal biomass.

17.3.1 Medium for Optimal Growth

The media used for fermentation greatly influences the nutritional requirements as well as physiochemical environment and thus directly affects productivity and process economics (Zhang and Greasham 1999; Bagde et al. 2010). Therefore, a suitable medium should invariably support vegetative growth and production of spores. The optimum growth conditions are observed in a modified Kaefer media containing (50 mg/100 mL) zinc oxide nanomaterial with peptone, 3.0; yeast extract, 3.0; KH₂PO₄, 1.83; and MgSO₄·7H₂O, 0.65 g/L. The concentration of other components was the same as in the original Kaefer medium without NaNO₃ and KCl, while the glucose concentration was 20 g/L (Kumar et al. 2011).

17.3.2 Sterilization of the Fermenter

Prior to the initiation of the production process, the fermenter needs to be sterilized. The fermentation media and the fermenter can be sterilized together or separately. The fermenter is sterilized by channeling steam into the vessel *via* all entries and releasing the steam slowly through air outlet. The jackets or coils of the fermenter are sterilized by heating them with steam. Also the steam pressure is maintained at 15 psi inside the vessel for 20 min approximately for thorough sterilization.

17.3.3 Cultivation in Bioreactor

For nanoembedded fungal biomass production in bioreactor, an active 2% inoculum raised in an optimized medium is used. The initial pH is calibrated at 6.4. As the biomass production is initiated, there is an uptake of glucose which decreases the pH to between 5.5 and 6.0 in late log phase. Since the optimum pH for sustainable growth of nanomaterial-infused *P. indica* is 5.8, there is no requirement for pH control in fermenter systems where the fungal cultures are grown on media containing complex nitrogen sources. The temperature range is in between 20 and 35 °C. However, for optimized growth the fungal cultures are grown at a temperature of 30 °C. The fungus grows best at lower agitation and low oxygen concentrations (Varma et al. 2001). Thus, the cultures are grown at 200 rpm and 20% working volume.

17.3.4 Recovery of Biomass Produced

After the desired biomass is obtained, the production process is terminated. The biomass produced in the fermenter vessel is removed. The produced biomass is then filtered, separating the filtrate from the biomass. After separation the biomass obtained is then formulated by mixing with sterilized magnesium sulfite, talcum powder, or vermiculite.

17.4 Cultivation in Bioreactor

Growth may be defined as an irreversible increase in the volume of an organism, usually accompanied by an increase in biomass. Mycelial fungi exhibit extension growth of hyphae, accompanied by an increase in biomass. Unicellular fungi (e.g., yeasts) may exhibit an increase in individual cell volume, accompanied by an increase in biomass. But collectively, the number of yeast cells within a culture

(i.e., cell concentration) may also increase, resulting in an increase in biomass of the culture as a whole.

17.4.1 Batch Fermentations

A tank of fermenter is filled with the prepared media to be fermented. The temperature and pH for microbial fermentation is properly adjusted, and occasionally nutritive supplements are added to the prepared media. The media is steam-sterilized in a pure culture process. The inoculum of a pure culture is added to the fermenter, from a separate pure culture vessel. Fermentation proceeds, and after the proper time, the contents of the fermenter are taken out for further processing. The fermenter is cleaned and the process is repeated. Thus, each fermentation is a discontinuous process divided into batches.

17.4.2 Mass Cultivation of *P. indica* with Nanomaterial in 7 L Fermenter

P. indica, which mimics AMF, represents a model system to understand the molecular basis of photo and mycobiont interaction. Its application in horticulture or agriculture as a potent biofertilizer and biocontrol agent is economically and practically feasible through the easy propagation of a fungal inoculum using liquid or axenic cultures.

It is shown that the fungus can be grown axenically on different synthetic media. Among the tested media, the best growth reported to be on Hill and Kaefer medium (2001) which is reported from different authors (Varma et al. 1999, 2001; Pham et al. 2004; Qiang et al. 2011). However, significant quantitative and morphological changes are detected when the fungus is grown on different nutrient composition with no apparent negative effect on plants (Kumar et al. 2011).

A 7 L bioreactor was used to grow *P. indica* on optimized Hill and Kaefer medium containing nanomaterial to establish the best conditions for a maximal biomass and spore production for scale-up studies.

When *P. indica* was grown in 7 L bioreactor on optimized Hill and Kaefer medium (containing 20.0 g/L glucose, 1.0 g/L peptone, 1.0 g/L yeast extract, 1.0 g/L Casein acid hydrolysate, 50.0 mL/L macroelement, 2.5 mL/L microelement stock solution, 1.0 mL/L vitamin stock solution, 1 mL/L CaCl₂, 0.1 M, 1.0 mL/L FeCl₃, and 50 mg/100 mL ZnO nanomaterial), a maximum dry cell weight of 8.45 g/L was obtained after 42 h of growth. The value of biomass yield and the specific daily growth rate were 0.87 and 2.05, respectively. The fungus initiated the sporulation after 48 h, and a spore yield of 9.25×10^9 spores/mL was achieved after 60 h of growth which is much higher in comparison to control. The early sporulation in this

case may be due to the varying and size-dependent interaction between the surface of the respective nanoparticle and the cell wall which has, e.g., an influence on the diffusion of nutrients and therefore affects the growth rate (Feng et al. 2013; Ren et al. 2011). Due to more efficient mixing and homogenized fungal suspension, the growth of fungus was faster in the bioreactor and resulted in early depletion of the carbon source and thereby early sporulation compared to a shake flask. A complete growth profile of *P. indica* on modified Hill and Kaefer medium has been depicted. The pattern of pH profile was quite similar in all these experiments where complex nitrogen sources were present in the growth medium. The uptake of glucose caused a decrease in pH of fermentation broth which might be due to the generation of acidic metabolites. The growth of fungus remained unaffected as long as the pH during the log phase was not reduced below 4.5.

17.4.3 Measurement of Cell Growth, Growth Yield, and Specific Growth Rate

The growth of *P. indica* was expressed in terms of dry cell weight (DCW) per liter of culture broth, which was determined by filtering a known volume of culture broth through Whatman No. 1 filter paper, drying to a constant weight in vacuum oven at 60 °C for about 48 h and weighing the dry mass. It was found that there was about 50% increase in dry cell weight of treated *P. indica* in comparison to control. Growth yield (YX/S) was calculated as grams of biomass produced per gram of substrate consumed. The specific growth rate (μ) was calculated from the equation $= 1/X \times dx/dt$, where X is the biomass concentration (g/L) at time t .

17.4.4 Measurement of Spores

P. indica produced pear-shaped chlamydospores, which were attached to the mycelium (Siddhantha et al. 2016). The spores were dislodged by adding 1 mL of Tween 80 to 100 mL of culture broth, vortexing, grinding in a mixer grinder, and sonicating for 5 min each. After their detachment, the spores were counted with a hemocytometer, and it was found that spore size was bigger in nanomaterial-treated culture as compared to control.

17.5 Conclusions

There was a significant increase, i.e., two- to threefold in fungal biomass in the presence of nanomaterial as compared to control using batch bioreactor for its mass cultivation. The fresh biomass was maximum in case of ZnO nanomaterial-infused medium. Colony morphology also differed; the medium appears useful for economical mass production of spore-rich *P. indica* biomass for agricultural and horticultural applications.

Acknowledgment Authors are thankful to DBT-SBIRI, DBT, and DST for providing confocal microscope facilities.

References

- Aziz N, Faraz M, Pandey R, Sakir M, Fatma T, Varma A, Barman I, Prasad R (2015) Facile algae-derived route to biogenic silver nanoparticles: synthesis, antibacterial and photocatalytic properties. *Langmuir* 31:11605–11612
- Aziz N, Pandey R, Barman I, Prasad R (2016) Leveraging the attributes of *Mucor hiemalis*-derived silver nanoparticles for a synergistic broad-spectrum antimicrobial platform. *Front Microbiol* 7:1984. doi:10.3389/fmicb.2016.01984
- Bagde US, Prasad R, Varma A (2010) Mass cultivation of *Piriformospora indica* in new Brunswick fermenter and its formulation as biofertilizer. *Asian J Microbiol Biotechnol Environ Sci* 12:911–916
- Bhuyan T, Khanuja M, Sharma R, Patel S, Reddy MR, Anand S, Varma A (2015a) A comparative study of pure and copper (Cu) doped ZnO nanorods for antibacterial and photocatalytic applications with their mechanism of action. *JNR* 17:288. doi:10.1007/s11051-015-3093-3
- Bhuyan T, Mishra K, Khanuja M, Prasad R, Varma A (2015b) Biosynthesis of zinc oxide nanoparticles from *Azadirachta indica* for antibacterial and photocatalytic applications. *Mater Sci Semicond Process* 32:55–61
- Feng Y, Cui X, He S, Dong G, Chen M, Wang J (2013) The role of metal nanoparticles in influencing arbuscular mycorrhizal fungi effects on plant growth. *Environ Sci Technol* 47:9496–9504
- Hatamie A, Khan A, Golabi M, Turner AP, Beni V, Mak WC, Sadollahkhani A, Alnoor H, Zargar B, Bano S, Nur O, Willander M (2015) Zinc oxide nanostructure-modified textile and its application to biosensing, photocatalysis, and as antibacterial material. *Langmuir* 31:10913–10921
- Hill TW, Käfer E (2001) Improved protocols for aspergillus medium: trace elements and minimum medium salt stock solutions. *Fungal Genet News Lett* 48:20–21
- Kumar V, Sahai V, Bisaria VS (2011) High-density spore production of *Piriformospora indica*, a plant growth promoting endophyte, by optimization of nutritional and cultural parameters. *Bioresour Technol* 102:3169–3175
- Murray CB, Kagan CR, Bawendi MG (2000) Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies. *Annu Rev Mater Sci* 30:546–610
- Pham GH, Kumari R, Singh A, Malla A, Prasad R, Sachdev M, Kaldorf M, Buscot F, Oelmüller R, Hampp R, Kumar SA, Karl-Heinz R, Gerhard K, Varma A (2004) Axenic culture of symbiotic fungus *Piriformospora indica*. *Plant Surf Microbiol* 68:593–613
- Prasad R, Pham HG, Kumari R, Singh A, Yadav V, Sachdev M, Garg AP, Peskan T, Hehl S, Sherameti I, Oelmüller R, Varma A (2005) Sebacinaceae: culturable mycorrhiza-like

- endosymbiotic fungi and their interaction with non-transformed and transformed roots. In: Declerck S, Strullu DG, Fortin A (eds) *Soil biology*. 4:291–313
- Prasad R, Kamal S, Sharma PK, Oelmueller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plants morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. *J Basic Microbiol* 53:1016–1024
- Prasad R, Pandey R, Barman I (2016) Engineering tailored nanoparticles with microbes: quo vadis. *WIREs Nanomed Nanobiotechnol* 8:316–330. doi:[10.1002/wnan.1363](https://doi.org/10.1002/wnan.1363)
- Qiang WM, Kogel KH, Schäfer P (2011) *Piriformospora indica*-a mutualistic basidiomycete with an exceptionally large plant host range. *Mol Plant Pathol* 13:508–518
- Raffi M, Hussain F, Bhatti TM, Akhter JJ, Hameed A, Hasan MM (2008) Antibacterial characterization of silver nanoparticles against *E coli* ATCC-15224. *J Mater Sci Technol* 24:192–196
- Ren HX, Liu L, Liu C, He SY, Huang J, Li JL (2011) Physiological investigation of magnetic iron oxide nanoparticles towards Chinese Mung Bean. *J Biomed Nanotechnol* 7:677–684
- Siddhanta S, Paidi SK, Bushley K, Prasad R, Barman I (2016) Exploring morphological and biochemical linkages in fungal growth with label-free light sheet microscopy and Raman spectroscopy. *ChemPhysChem*. doi:[10.1002/cphc.201601062](https://doi.org/10.1002/cphc.201601062)
- Suman, Prasad R, Jain VK, Varma A (2010) Role of nanomaterials in symbiotic fungus growth enhancement. *Curr Sci* 99:1189–1191
- Taton TA (2002) Nanostructures as tailored biological probes. *Trends Biotechnol* 20:277–279
- Varma A, Verma S, Sudha NS, Bütehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Appl Environ Microbiol* 65:2741–2744
- Varma A, Singh A, Sudha NS, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Hurek T, Bleichert O, Rexer KH, Kost G, Hahn A, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica*: an axenically culturable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) *Fungal associations, The mycota*, vol IX. Springer, Berlin, pp 125–150
- Varma A, Jain VK, Suman, Prasad R (2015) A nanomaterial based culture medium for microbial growth enhancement (Application Number: 14/DEL/2009; Patent Number: 267958)
- Whitesides GM (2003) The right size in nanobiotechnology. *Nat Biotechnol* 21:1161–1165
- Zhang J, Greasham R (1999) Chemically defined media for commercial fermentations. *Appl Microbiol Biotechnol* 51:407–421

Chapter 18

Understanding the Mycorrhiza-Nanoparticles Interaction

Avinash Ingle, Dnyaneshwar Rathod, Ajit Varma, and Mahendra Rai

Abstract Arbuscular mycorrhizal fungi (AMF) always associate with the roots of higher plants and form a mutualistic symbiosis with the roots of over 90% of plant species, including forest trees, wild grasses, and many crops. Recently, considerable efforts were put to revolutionize agricultural systems through the applications of nanotechnology in various ways. Nanoparticles exploited for plant growth promotion showed the controversial opinions. Similarly, the interaction of various nanoparticles with mycorrhizal fungi found to influence its growth and showed both positive and negative effects. Some of the nanoparticles helps in colonization of AMF, whereas some negatively affects the colonization. Therefore, understanding the exact mechanism of interaction between AMF and nanoparticles is necessary.

Hence, in this book chapter, we have focused on the influence of different nanoparticles on the growth of AMF. Moreover, we have also discussed the role of nanoparticle in plant growth promotion.

18.1 Introduction

Arbuscular mycorrhizal fungi (AMF) are the most widespread fungal symbionts of plants, being associated with more than 90% of current land plants (Bonfante and Genre 2010; Prasad et al. 2017a). All AMF belong to the phylum *Glomeromycota*, a monophyletic group that diverged from the same common ancestor as *Ascomycota* and *Basidiomycota* (Prasad et al. 2017a). AMF are common soil microbes whose association with roots can have extensive effects on growth of the host plant (Rathod et al. 2011). AMF associations enhance not only the uptake of nutrients and water but also increase the resistance of their host plant towards disease and drought. AMF-infected roots are less susceptible to certain types of pathogens. Therefore, in

A. Ingle • D. Rathod • M. Rai (✉)

Nanobiotechnology Laboratory, Department of Biotechnology, SGB Amravati University,
Amravati 444602, Maharashtra, India
e-mail: mkrai123@rediffmail.com

A. Varma

Amity Institute of Microbial Technology, Amity University, E-3 Block, Fourth Floor, Sector
125, Noida 201303, Uttar Pradesh, India

recent years, major interest has centered on AMF in the control of soilborne pathogens present in the rhizospheric region of plants. Many researchers reported that colonization of roots by AMF confers resistance to plants against invasion by pathogens (Caron et al. 1985; Prasad 1998; Schouteden et al. 2015). The AM fungal hyphae extend into the rhizosphere and improve the absorption of water and nutrients such as phosphate and nitrogen from the soil through arbuscules (Chalot et al. 2006; Prasad et al. 2017a).

Recently, tremendous efforts have been made by the researchers all over the world to revolutionize agricultural systems through the applications of nanotechnology. Working with the nanomaterials in nanotechnology raises hope for improving agricultural productivity by encountering challenges which are unsolved conventionally. Moreover, various nanomaterials such as nanoclays and zeolites can be used to increase the efficiency of applied fertilizer, and it also helps in restoration of soil fertility by releasing fixed nutrients (Manjunatha et al. 2016). Apart from this, various studies have been performed to demonstrate the efficacy of different metal nanoparticles in plant growth promotion, whereas the findings reported showed that nanoparticles can exert both positive and negative effects on plant growth. However, the efficacy of nanoparticles is found dependent on various parameters like concentration, size, and shape of nanoparticles (Yin et al. 2012; Syu et al. 2014). In addition, effect of nanoparticles may vary plant to plant and even in different species of the same genus.

Nanoparticles are also reported to have great influence on the growth of AMF and other soil microorganisms beneficial for plants which are generally present in soil. Primary studies showed that direct application of metal nanoparticles exert both positive (Suman et al. 2010; Feng et al. 2013) and adverse effects (Cao et al. 2016) on the growth of AMF which are colonized in the plant roots due to their accumulation. Hence, there is a need to understand the exact interaction of nanoparticles and AMF in plants.

Therefore, the main aim of the chapter is to focus on understating of interaction of AMF and different nanoparticles. Moreover, we have also discussed the most contradictory and important area of research, i.e., role of nanoparticles in plant growth promotion. Apart from this, the role of endo- and ectomycorrhizal fungi has been discussed.

18.2 Plant and Microbe Symbiosis

Arbuscular mycorrhizae are characterized by the formation of unique structures, arbuscules, and vesicles by fungi of the phylum *Glomeromycota*. AMF develop a mutualistic relationship with the roots of host plants. AMF help plants to uptake nutrients such as nitrogen, phosphorus, and sulfur from the soil. It is reported that the development of the arbuscular mycorrhizal symbiosis plays a crucial role in initial colonization of land by plants and evolution of the vascular plants (Brundrett 2002; Prasad et al. 2017a).

A minor group of fungi, the parasitic and mutualistic symbionts, feed on living organisms. Such a classification cannot be easily applied to mycorrhizal fungi, a heterogeneous group of species spread over diverse fungal taxa. AMF are always associated with the roots of higher plants; indeed over 90% of plant species, including forest trees, wild grasses, and many crops (Bonfante and Genre 2010). Therefore, both partners are benefited from the mutual relationship, that is, mycorrhizal fungi improve the nutrient status of their host plants, influencing mineral nutrition, water absorption, growth, and disease resistance, whereas in exchange, the host plant is necessary for fungal growth and reproduction.

Substantial progress has been made in exploring the use of microorganisms in control of plant diseases in integrated plant disease management. One such strategy is the better exploitation of microbes present in soil, which contribute to soil fertility. AMF are ubiquitous in nature and constitute an integral component of terrestrial ecosystems, forming symbiotic associations with plant root systems of over 80% of all terrestrial plant species, including many agronomically important species (Berruti et al. 2016). Therefore, AMF are considered natural biofertilizers, because they provide the host with water, nutrients, and pathogen protection, in exchange for photosynthetic products. AMF are particularly important in organic and or sustainable farming systems that rely on biological processes rather than agrochemicals to control plant diseases (Harrier and Watson 2004). Significant advances have been made in the last two decades to understand the potential of mycorrhizal fungi in suppression of plant pathogens especially soilborne pathogens in wide range of fruits and vegetable host plants (Naqvi and Naqvi 2007). Plants are generally affected by many pathogens, which cause disease to the plants and thereby reduce the crop productivity, but the plants which are inoculated with mycorrhiza exhibit increased resistance to the fungal root-rot disease (Cameron et al. 2013; George et al. 2016). Mycorrhizae enter into a mutualistic relationship with plant roots, in which the fungi actually become integrated into the physical structure of the roots. The fungus derives nutritional uptake from the plant roots, without causing any plant disease.

18.3 Mycorrhiza: As Plant Symbionts

Mycorrhizal fungi have a close symbiotic relationship with plant roots. Mycorrhizal fungi colonize the plant's root system and develop a symbiotic association called "mycorrhiza." The terms symbiotic and mutualistic have been used interchangeably to describe mycorrhizal associations (Kaur et al. 2014). There are two major types of mycorrhizal fungi, that is, endomycorrhizal and ectomycorrhizal fungi. Both groups play an important role in symbiotic association relationship between host and AMF. Ectomycorrhizae basically originate on trees and form visible structures. These fungi colonize in trees as well as shrubs and most herbaceous plants and do not form visible structures (Kaur et al. 2014).

Among the types of endomycorrhizal fungi, arbuscular mycorrhizal (AM) fungi are the most widespread in soils. The most important members of endomycorrhizal group are called arbuscular mycorrhizae (AM). Earlier AMF were also called as vesicular arbuscular mycorrhizae (VAM) as fungal hyphae insert into the cortical root cell wall and once inside the plant cell, form small hyphae branched structures known as arbuscules. This name is derived from the occurrence of two types of structure characteristics of the fungi which belong to the family *Endogonaceae*, i.e., arbuscules (arbuscules are finely branched structures that form within a cell and serve as a major metabolic exchange site between the plant and the fungus) and vesicles (sac-like structures, emerging from hyphae, which serve as storage organs for lipids). The endomycorrhizal fungi involved consist of septate hyphae which are members of the phycmycetes and basidiomycetes. The hyphae of fungi penetrate the cells of the root cortex forming an internal hyphae network. Numbers of plants including many agricultural crops have infection by fungi that are vesicular arbuscular mycorrhizae. The actual process for root colonization by AMF starts with germination of resting spores present in the soil after subjected to the favorable conditions which is followed by the production of a short explorative mycelium. The perception of plant secretions, released by the host root, induces recursive hyphal branching, increasing the probability of a direct contact between the symbionts. Meanwhile, fungal secretions are perceived by the root, where they trigger calcium spiking through the activation of the common symbiosis pathway. This symbiotic reaction leads to signal transduction which activates cellular and transcriptional responses (green cells and nuclei). The interaction between the plant and fungus is followed by the adhesion of a hyphopodium to the root surface. This stimulates the assembly of a broad aggregation of cytoplasm (yellow), named the prepenetration apparatus (PPA) in the contacted epidermal cell and underlying outer cortical cell. Subsequent intracellular fungal colonization strictly follows the route of PPAs from the epidermis to the inner cortex. Here, intercellular hyphae can develop along the root axis. The PPA mechanism is then replicated in the contacted inner cortical cells, both before fungal entry and on a smaller scale branching (Fig. 18.1).

AMF significantly enhanced the potential of plants to absorb phosphorus (P) and other nutrients that are relatively immobile and available in low concentration in the soil (Kaur et al. 2014). It also plays a significant role in P nutrition of crop. Schüßler et al. (2001) reported that AMF are obligate symbionts belonging to the phylum *Glomeromycota*. Smith and Smith (2011) stated that AMF provides mineral nutrients to the host plant in exchange for photosynthetic products from the host. AMF are potential to improve remarkably plant mineral nutrient gaining, mostly in low-nutrient conditions, and it has clearly been studied that plants retain a symbiotic P uptake pathway (Tawaraya 2003; Smith and Smith 2011; Berruti et al. 2016).

Ectomycorrhizal fungi (EMF) are mostly observed in forest ecosystems and also found in other natural environments. EMF grow between root cells without piercing them. The fungal hyphae grow outer side in thick manner called as fungal mantle

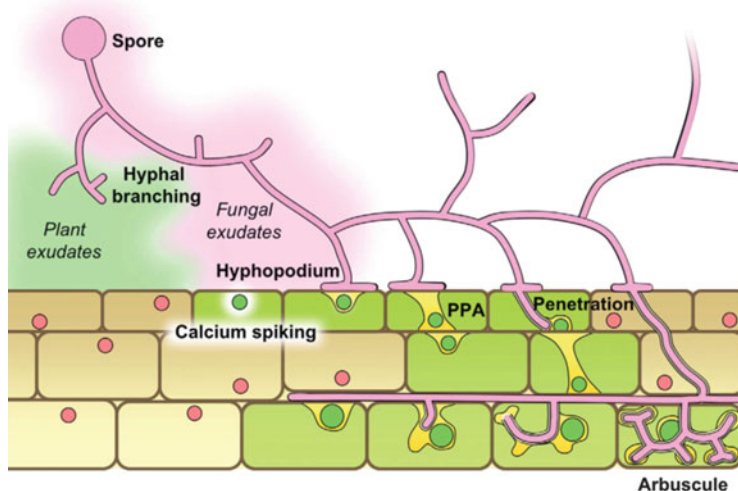


Fig. 18.1 Schematic summary of the root colonization process by AMF [Reused from Bonfante and Genre (2010) with copyright permission from Nature Publishing Group]

which forms symbiotic relationship between plants and fungi. The EMF surrounds the root tip with a thick mantle of closely appressed hyphae, whereas the Hartig net develops around epidermal cells (green). In the case of arbuscular mycorrhizae, the root tip is usually not colonized. Hyphae develop from a spore and produce a hyphopodium on the root epidermis. Intraradical colonization proceeds both intra- and intercellularly and culminates with the formation of arbuscules, little fungal trees, and inside inner cortical cells (brown) (Fig. 18.2).

18.4 Role of Nanoparticles in Plant Growth

Nanotechnology has opened large scope of novel application in the various fields including agricultural sector, because the building blocks of nanotechnology, i.e., nanoparticles, have novel and unique physicochemical and biological properties. Various studies carried out in the past reported the potential use of some metal nanoparticles as nano-herbicides, nano-pesticides, and nano-fertilizers (Rai and Ingle 2012; Prasad et al. 2017b). In some cases, these nanomaterials were used as vehicle for the target-specific delivery in plants. Moreover, recently many researchers around the globe are focusing on one of the important applications of various nanoparticles, i.e., their role in plant growth promotion. In spite of the plenty of information available on the toxicity of nanoparticles to plant system, few studies have been conducted, and depending upon these studies carried out, there are distinct opinions (both positive and negative effects) of researchers regarding the role of nanoparticles in plant growth and development (Siddiqui et al. 2015).

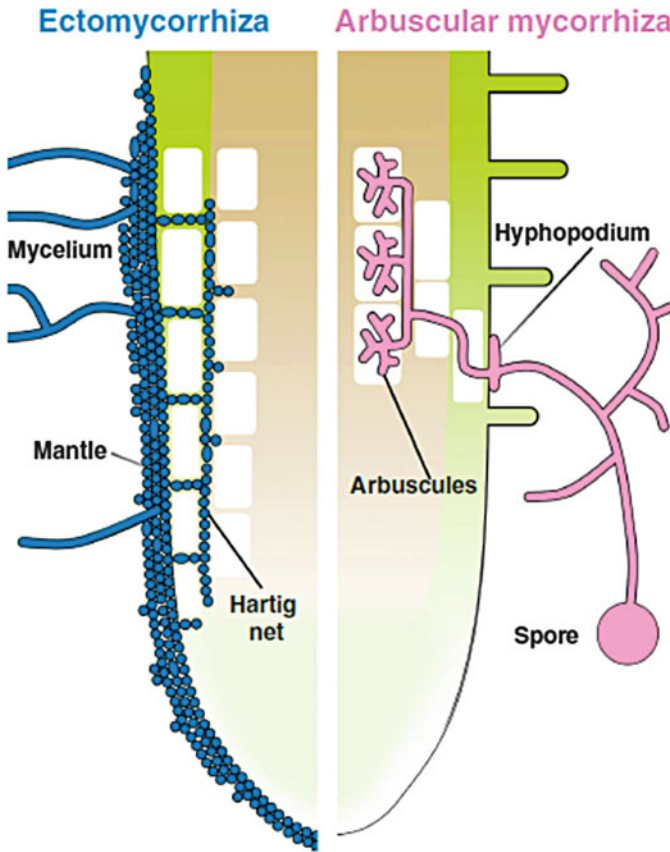


Fig. 18.2 Schematic representation of root colonization structures in ectomycorrhizal (blue) and arbuscular mycorrhizal (pink) interactions [Reused from Bonfante and Genre (2010) with copyright permission from Nature Publishing Group]

Generally, germination of seeds is the first step toward the plant growth and development which is later followed by root elongation and shoot formation. Hence, the nanoparticles which help in the development of these parts will show positive effect in plant growth promotion. However, those which don't support the growth of these parts exert negative effects on plant growth. In this context, various studies have been performed which exploit different nanoparticles. Some of these studies are briefly discussed here.

Siddiqui and Al-Wahaibi (2014) demonstrated the seed germination efficacy of silicon dioxide nanoparticles (SiO_2NPs) in tomato seeds (*Lycopersicon esculentum*). This was evident by studying various parameters such as percent seed germination, mean germination time, seed germination index, seed vigor index, fresh weight and dry weight of seedling, etc. The collective results obtained suggested that SiO_2NPs showed enhanced seed germination at lower concentration

(i.e., 8 g/L). In another study, Suriyaprabha et al. (2012) reported significant increase in seed germination in maize plant after application of SiO₂NPs due to sufficient availability of nutrients in the presence of SiO₂NPs. SiO₂NPs also reported to have improved seedling growth. Bao-shan et al. (2004) studied the effect of SiO₂NPs on the growth of seedlings in Changbai larch (*Larix olgensis*). The results showed the significant increase in the seedling growth which was confirmed from the improved mean height, root collar diameter, main root length, and the number of lateral roots of seedlings and also induced the synthesis of chlorophyll.

Similarly, there are many reports on zinc oxide nanoparticles (ZnONPs) which proved their ability in plant growth promotion in lower concentration; however, higher concentration exerts negative effects by impairing the seed germinations, and it also varies from plant to plant (de la Rosa et al. 2013; Raskar and Laware 2014). These include studies of Prasad et al. (2012) in peanut, Sedghi et al. (2013) in soybean, and Ramesh et al. (2014) in wheat. Moreover, studies carried out by Raliya and Tarafdar (2013) and Mahajan et al. (2011) demonstrated that ZnONPs showed significant growth and development in shoot and root length and biomass of *Cyamopsis tetragonoloba*, *Vigna radiata*, and *Cicer arietinum* plants, respectively. However, in another study, it was reported that ZnONPs do not show any improved efficacy in all parameters (viz., seed germination percentage, root length, and number of roots) studied in rice (*Oryza sativa* L.) (Boonyanitipong et al. 2011). They report stunt root length and reduce the number of roots and also detrimental effects on rice roots at early seedling stage.

Apart from these, other metal nanoparticles like gold (AuNPs) also reported to have positive effect on seed germination. Barrena et al. (2009), Arora et al. (2012), Savithramma et al. (2012), and Gopinath et al. (2014) demonstrated the increase in seed germination in lettuce and cucumber, *Brassica juncea*, *Boswellia ovalifoliolata*, and *Gloriosa superba*, respectively. Not only seed germination AuNPs also showed improved growth and development of other plant parts (Arora et al. 2012; Gopinath et al. 2014). On the contrary, Shah and Belozerovala (2009) reported that AuNPs exert toxic effects on the various proteins which help in transportation of wide range of molecules including water, thereby interfering with aquaporin function of plant.

Like AuNPs, silver nanoparticles (AgNPs) are also found to have significant effects on seed germination of different plants including *Bacopa monnieri* (Krishnaraj et al. 2012) and *B. ovalifoliolata* (Savithramma et al. 2012). In addition, increased plant growth profile (shoot and root length, leaf area) was reported in *B. juncea* (Sharma et al. 2012). Various physical parameters such as concentration, shape, and size of AgNPs play key a role in the plant growth promotion (Syu et al. 2014). Recently, Razzaq et al. (2016) demonstrated the effect of different concentrations (0, 25, 50, 100, and 150 ppm) of AgNPs on growth of wheat plants. The results showed that plants treated with 25 ppm AgNPs showed more prominent growth. Apart from this, its effect was also found to be dependent on plants and their species (Yin et al. 2012). In contradiction to above studies, Gruyer et al. (2013) reported negative effect on root elongation of lettuce. Other nanoparticles like

titanium dioxide (TiO₂NPs) also showed improved seed germination and also promoted growth of radicle and plumule of canola seedlings (Mahmoodzadeh et al. 2013). Jaberzadeh et al. (2013) demonstrated that TiO₂NPs help in growth promotion of wheat plant growth and increased yield was reported even in water deficit stress condition.

In addition, studies carried out on the use of carbon nanotubes (CNTs) in plant growth and development showed both positive as well as negative effects. The reports by Gajanan et al. (2010), Mondal et al. (2011), Morla et al. (2011), and Nalwade and Neharkar (2013) demonstrated that multi-walled carbon nanotubes (MWCNTs) showed high germination rate in tomato, hybrid Bt cotton, *B. juncea*, *Phaseolus mungo*, and rice. However, Husen and Siddiqi (2014) and Lin and Xing (2007) reported that MWCNTs do not exhibit a positive influence on seed germination.

Overall, nanoparticles discussed above showed both positive and negative effects on the plant growth. Some of these play a key role in the growth promotion, and some of these are reported to exert toxic effects on plants. On one hand, the significant increase in seed germination and other parameters may be effective to increase the crop yield. On the other hand, direct exposure of plants to nanoparticles causes significant phytotoxicity (Tripathi et al. 2017). Hence, extensive care needs to be taken during the disposal of wastes containing nanoparticles, and also there is huge need to encourage the studies on toxicity assessment and impacts of nanoparticles on agricultural and environmental systems.

18.5 Mycorrhiza: Nanoparticle Interaction

It is a well-known fact that AMF are one of the most important members of soil microbial community which can form a mutualistic symbiosis with the roots of over 90% of land plants. AMF play key role in plant growth promotion directly or indirectly. It helps in proper aquaporin function (water uptake) of plant and uptake of many other nutrients required for plant growth. On the other side, applications of different nanoparticles in agriculture are increasing day by day; however, there are different positive and negative opinions of researchers about the role of nanoparticles in plant growth. Moreover, some of the attempts have been made to alleviate the negative effects of nanoparticles by using AMF. But, still there is no sufficient literature available regarding the studies on interaction of mycorrhizal fungi and nanoparticles.

As described earlier, direct application of some of the metal nanoparticles exerts toxic effects by accumulating in various parts of plants when used in higher concentration. Application of nanoparticles in higher doses also contaminate the soil which ultimately effects on crop yield and harms beneficial soil microorganisms, posing new concerns and challenges. According to the recent study, it was observed that the interaction of AMF with such nanoparticles help in the alleviation of negative effects exerted in plants. Wang et al. (2016) performed some

experiments, in which they demonstrated the interactions between maize plants treated with ZnONPs and inoculated with or without AMF. They reported that when the maize plants were treated with ZnONPs at the concentration of 800 mg/kg, decreased plant mineral nutrient acquisition, photosynthetic pigment concentrations, and root activity which were observed in plants without AMF. However, plants inoculated with AMF showed increased growth, nutrient uptake, photosynthetic pigment content, and superoxide dismutase activity in leaves. It is because of interaction of AMF with nanoparticles which helps in the decrease of Zn bioavailability and accumulation, thereby increasing mineral nutrients and antioxidant capacity of plant.

Moreover, available reports suggested that interaction of some nanoparticles with AMF helps in its colonization in plant roots and associated soil. Feng et al. (2013) studied the biological effects of AgNPs and iron oxide nanoparticles (FeONPs) and Fe₂O₃ and Ag in bulk material form on AMF in mycorrhizal clover (*Trifolium repens*) in dose-dependent manner. The results demonstrated the significant increase in AMF growth and function reported in plant treated with nanoparticles; however, their respective bulk material did not colonize AMF in plants. Improved colonization of AMF in plant roots and associated soil will automatically reflect in improved plant growth. On the contrary, Dubchak et al. (2010) evaluated the efficacy of AgNPs and TiNPs on the development of colonization of mycorrhiza in *Helianthus annuus* cultivated in the presence of radioactive ¹³⁴Cs. It was reported that mycorrhizal colonization and uptake of ¹³⁴Cs were greatly affected in the presence of AgNPs and TiNPs. However, application of activated carbon reduced the effect of these nanoparticles and increases both colonization of AMF and uptake of ¹³⁴Cs by plants.

Li et al. (2015) also reported exactly opposite results in case of ZnONPs and ZnSO₄ (bulk form of Zn). In their study, biological effects of ZnONPs and ZnSO₄ alone and in combination on colonization of AMF (*Funneliformis mosseae*) in maize plants were studied. They reported that both ZnONPs and ZnSO₄ at the concentration of 500 mg/kg inhibited the AMF colonization and also the growth of maize plants, whereas improved colonization of AMF and growth of plants were reported when they were used in combination. It may be due to the decreased Zn concentrations and improved uptake of other nutrients in maize plants.

It is well documented that AMF not only itself help in plant growth promotion but also help to increase the activity of other soil microorganisms. In this context, recently, Cao et al. (2016) demonstrated the interaction of AMF and iron oxide nanoparticles (Fe₃O₄NPs) and studied the role of AMF in the alleviation of negative effects of these nanoparticles on other microbes present in rhizospheric soils. The results obtained revealed that Fe₃O₄NPs in higher concentration exert toxic effects which leads to significant decrease in soil bacterial abundance which further leads to decrease in soil-dissolved organic contents. However, no significant changes were reported in soil bacterial abundance and soil-dissolved organic after Fe₃O₄NPs treatment in the presence of AMF. It indicated that AMF alter the effects of Fe₃O₄NPs on soil microorganisms, possibly by influencing plant growth and organic matter released from plant roots.

Contamination of soil by various types of nanoparticles is a great challenge. Interaction of metal nanoparticles having broad-spectrum antimicrobial activities with soil microbes including various mycorrhizal fungi resulted in the depletion of useful microflora of soil (Aziz et al. 2016). Sweet and Singleton (2015) studied the effects of various concentrations of AgNPs (0, 350, and 790 mg/kg) on the growth of EMF which are equally important and responsible for enhancing growth of plants by nutrient transfer like AMF and on growth of pine plant. It was reported that AgNPs even at lower concentration, i.e., 350 mg/kg, showed significant reduction in growth of EMF in pine root and also it affects the fresh root and shoot biomass in pine plants.

The efficacy of TiO₂NPs was studied on the growth of maize and soybean plants and associated microbial community including AMF. The results revealed that there were no any significant effects on plant growth and the composition of bacterial communities within the rhizosphere. However, composition of AMF was greatly affected in the presence of these nanoparticles (Burke et al. 2014). The possible reason behind the interaction maybe the increase in the concentration of titanium (Ti) in the roots of plants due to binding of TiO₂NPs to plant roots. The increased concentration of Ti in roots affects the composition of AMF (Seeger et al. 2009) as these are colonized in the interior of the root system. However, as the other bacterial community is mainly present in the rhizosphere, it was not affected much.

Although the various studies have been performed to understand the interaction between different nanoparticles and mycorrhizal fungi, due to huge contradiction in the finding reported for each study, researchers have to wait for some more time period to understand the exact role of different nanoparticles and their actual interaction with mycorrhizal fungi.

18.6 Conclusions

Considering the facts described in the present book chapter, it can be concluded that mycorrhizal fungi are the most important symbiotic agents for plants that help in growth promotion through the uptake of various nutrients and water. Although, many researchers claimed application of nanoparticles in agricultural systems including plant growth promotion, the data generated from the previous studies set a question mark on such claims. The findings reported for the same are contradictory; some showed positive effects of nanoparticles, whereas some others showed negative effects on plant growth. Similarly, there are mixed opinions, in the context of the interaction of mycorrhizal fungi and nanoparticles. On one hand, AMF are found to alleviate the negative effects of nanoparticles and play a key role in the management of environmental risks posed by different nanoparticles in agriculture, while on the other hand, some nanoparticles are found to affect the colonization of AMF in plant roots, thereby affecting plant growth. Hence, there is

requirement of extensive studies on interaction of mycorrhizal fungi and nanoparticles to understand the exact mechanism involved in it.

Acknowledgment Ajit Varma is thankful to DBT for partial funding and DST for providing confocal microscope. MKR thankfully acknowledges the financial help rendered by UGC, New Delhi, under Special Assistance Programme (DRS-I).

References

- Arora S, Sharma P, Kumar S, Nayan R, Khanna PK, Zaidi MGH (2012) Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. *Plant Growth Regul* 66:303–310
- Aziz N, Pandey R, Barman I, Prasad R (2016) Leveraging the attributes of *Mucor hiemalis*-derived silver nanoparticles for a synergistic broad-spectrum antimicrobial platform. *Front Microbiol* 7:1984. doi:10.3389/fmicb.2016.01984
- Bao-shan L, Shao-qi D, Chun-hui L, Li-jun F, Shu-chun Q, Min Y (2004) Effect of TMS (nanostructured silicon dioxide) on growth of Changbai larch seedlings. *J Forest Res* 15:138–140
- Barrena R, Casals E, Colón J, Font X, Sánchez A, Puentes V (2009) Evaluation of the ecotoxicity of model nanoparticles. *Chemosphere* 75:850–857
- Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Front Microbiol* 6:1559. doi:10.3389/fmicb.2015.01559
- Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nat Commun* 1:48. doi:10.1038/ncomms1046
- Boonyanitipong P, Kumar P, Kositsup B, Baruah S, Dutta J (2011). Effects of zinc oxide nanoparticles on roots of rice *Oryza Sativa* L. In: Proceeding of international conference on Environment and BioScience, vol 21. IACSIT Press, Singapore, pp 172–176
- Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. *New Phytol* 154:275–304
- Burke DJ, Zhu S, Publico-Lansigan MP, Hewins CR, Samia ACS (2014) Titanium oxide nanoparticle effects on composition of soil microbial communities and plant performance. *Biol Fertil Soils* 50:1169–1173
- Cameron DD, Neal AL, van Wees SCM, Ton J (2013) Mycorrhiza-induced resistance: more than the sum of its parts? *Trends Plant Sci* 18:539–545
- Cao J, Feng Y, Lin X, Wang J (2016) Arbuscular mycorrhizal fungi alleviate the negative effects of iron oxide nanoparticles on bacterial community in rhizospheric soils. *Front Environ Sci* 4:1–10. doi:10.3389/fenvs.2016.00010
- Caron M, Fortin JA, Richard C (1985) Effect of *Glomus intraradices* on infection by *Fusarium oxysporum* f. sp. *radicopersici* on tomato over a 12 week period. *Can J Bot* 64:552–556
- Chalot M, Blaudez D, Brun A (2006) Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface. *Trends Plant Sci* 11:263–266
- de la Rosa G, Lopez-Moreno ML, de Haro D, Botez CE, Peralta-Videa JR, Gardea-Torresdey JL (2013) Effects of ZnO nanoparticles in alfalfa, tomato, and cucumber at the germination stage: root development and X-ray absorption spectroscopy studies. *Pure Appl Chem* 85:2161–2174
- Dubchak S, Ogar A, Mietelski JW, Turnau K (2010) Influence of silver and titanium nanoparticles on arbuscular mycorrhiza colonization and accumulation of radio-caesium in *Helianthus annuus*. *Spanish J Agric Res* 8:103–108
- Feng Y, Cui X, He S, Dong G, Chen M, Wang J, Lin X (2013) The role of metal nanoparticles in influencing arbuscular mycorrhizal fungi effects on plant growth. *Environ Sci Technol* 47:9496–9504

- Gajanan G, Deuk SY, Donghee P, Sung LD (2010) Phytotoxicity of carbon nanotubes assessed by *Brassica Juncea* and *Phaseolus Mungo*. *J Nanoelectron Optoelectron* 5:157–160
- George TS, Dou D, Wang X (2016) Plant-microbe interactions: manipulating signals to enhance agricultural sustainability and environmental security. *Plant Growth Regul* 80:1–3
- Gopinath K, Gowri S, Karthika V, Arumugam A (2014) Green synthesis of gold nanoparticles from fruit extract of *Terminalia arjuna*, for the enhanced seed germination activity of *Gloriosa superba*. *J Nanostruct Chem* 4:1–11
- Gruyer N, Dorais M, Bastien C, Dassylva N, Triffault-Bouchet G (2013) Interaction between sliver nanoparticles and plant growth. International symposium on new technologies for environment control, energy-saving and crop production in greenhouse and plant factory–greensys, Jeju, Korea, 6–11 Oct 2013
- Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. *Pest Manag Sci* 60:149–157
- Husen A, Siddiqi KS (2014) Carbon and fullerene nanomaterials in plant system. *J Nanotechnol* 12:1–10
- Jaberzadeh A, Moaveni P, Moghadam HRT, Zahedi H (2013) Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. *Not Bot Horti Agrobot* 41:201–207
- Kaur R, Singh A, Kang JS (2014) Influence of different types mycorrhizal fungi on crop productivity. *Curr Agric Res J* 2:51–54
- Krishnaraj C, Jagan EG, Ramachandran R, Abirami SM, Mohan N, Kalaichelvan PT (2012) Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. *Plant growth metabolism. Process Biochem* 47:51–58
- Li S, Liu XQ, Wang FY, Miao YF (2015) Effects of ZnO nanoparticles, ZnSO₄ and arbuscular mycorrhizal fungus on the growth of maize. *Huan Jing KeXue* 36:4615–4622
- Lin D, Xing B (2007) Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environ Pollut* 150:243–250
- Mahajan P, Dhoke SK, Khanna AS (2011) Effect of nano-ZnO particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method. *J Nanotechnol*:1–7. doi:10.1155/2011/696535
- Mahmoodzadeh H, Nabavi M, Kashi H (2013) Effect of nanoscale titanium dioxide particles on the germination and growth of canola (*Brassica napus*). *J Ornamental Horti Plants* 3:25–32
- Manjunatha SB, Biradar DP, Aladakatti YR (2016) Nanotechnology and its applications in agriculture: a review. *J Farm Sci* 29:1–13
- Mondal A, Basu R, Das S, Nandy P (2011) Beneficial role of carbon nanotubes on mustard plant growth: an agricultural prospect. *J Nanopart Res* 13:4519–4528
- Morla S, Ramachandra Rao CSV, Chakrapani R (2011) Factors affecting seed germination and seedling growth of tomato plants cultured in vitro conditions. *J Chem Bio Phys Sci B* 1:328–334
- Nalwade AR, Neharkar SB (2013) Carbon nanotubes enhance the growth and yield of hybrid Bt cotton Var. ACH-177-2. *Int J Adv Sci Tech Res* 3:840–846
- Naqvi NS, Naqvi SAMH (2007) Mycorrhiza in management of fruits and vegetables diseases. *Dis Fruits Veg* 2:537–558
- Prasad K (1998) Biological control of rhizospheric microflora of *Saccharum officinarum* L. plants through vesicular arbuscular mycorrhizal (*Glomus fasciculatum*) fungi. *Biome* 8:131–136
- Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreepasad TSP, Sajanlal R, Pradeep T (2012) Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *J Plant Nutr* 35:905–927
- Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017a) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) *Mycorrhiza*. Springer, Cham, pp 1–7

- Prasad R, Bhattacharyya A, Nguyen QD (2017b) Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. *Front Microbiol* 8:1014. doi:[10.3389/fmicb.2017.01014](https://doi.org/10.3389/fmicb.2017.01014)
- Rai M, Ingle A (2012) Role of nanotechnology in agriculture with special reference to management of insect pests. *Appl Microbiol Biotechnol* 94:287–293
- Raliya R, Tarafdar JC (2013) ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in cluster bean (*Cyamopsis tetragonoloba* L.). *Agric Res* 2:48–57
- Ramesh M, Palanisamy K, Babu K, Sharma NK (2014) Effects of bulk & nano-titanium dioxide and zinc oxide on physio-morphological changes in *Triticum aestivum* Linn. *J Glob Biosci* 3:415–422
- Raskar SV, Laware SL (2014) Effect of zinc oxide nanoparticles on cytology and seed germination in onion. *Int J Curr Microbiol Appl Sci* 3:467–473
- Rathod DP, Brestic M, Shao HB (2011) Chlorophyll *a* fluorescence determines the drought resistance capabilities in two varieties of mycorrhized and non-mycorrhized *Glycine max* Linn. *Afr J Microbiol Res* 5:4197–4206
- Razzaq A, Ammara R, Jhanzab HM, Mahmood T, Hafeez A, Hussain S (2016) A novel nanomaterial to enhance growth and yield of wheat. *J Nanosci Technol* 2:55–58
- Savithamma N, Ankanna S, Bhumi G (2012) Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata* an endemic and endangered medicinal tree taxon. *Nano Vision* 2:61–68
- Schouteden N, Waele DD, Panis B, Vos CM (2015) Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: a review of the mechanisms involved. *Front Microbiol* 6:1280. doi:[10.3389/fmicb.2015.01280](https://doi.org/10.3389/fmicb.2015.01280)
- Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res* 105:1413–1421
- Sedghi M, Hadi M, Toluie SG (2013) Effect of nano zinc oxide on the germination of soybean seeds under drought stress. *Ann West Univ Timisoara Ser Biol XVI* 2:73–78
- Seeger EM, Baun A, Kanstner M, Trapp S (2009) Insignificant acute toxicity of TiO₂ nanoparticles to willow trees. *J Soils Sediments* 9:46–53
- Shah V, Belozerova I (2009) Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. *Water Air Soil Pollut* 197:143–148
- Sharma P, Bhatt D, Zaidi MG, Saradhi PP, Khanna PK, Arora S (2012) Silver nanoparticle mediated enhancement in growth and antioxidant status of *Brassica juncea*. *Appl Biochem Biotechnol* 167:2225–2233
- Siddiqui MH, Al-Wahaibi MH (2014) Role of nano-SiO₂ in germination of tomato (*Lycopersicon esculentum* seeds Mill.) *Saudi Biol Sci* 21:13–17
- Siddiqui MH, Al-Wahaibi MH, Firoz M, Al-Khaishany MY (2015) Role of nanoparticles in plants. In: Siddiqui MH, Al-Wahaibi MH, Firoz M (eds) *Nanotechnology and plant sciences*. Springer, Cham, pp 19–35
- Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Ann Rev Plant Biol* 62:227–250
- Suman, Prasad R, Jain VK, Varma A (2010) Role of nanomaterials in symbiotic fungus growth enhancement. *Curr Sci* 99:1189–1191
- Suriyaprabha R, Karunakaran G, Yuvakkumar R, Rajendran V, Kannan N (2012) Silica nanoparticles for increased silica availability in maize (*Zea mays* L) seeds under hydroponic conditions. *Curr Nanosci* 8:902–908
- Sweet MJ, Singleton I (2015) Soil contamination with silver nanoparticles reduces Bishop pine growth and ectomycorrhizal diversity on pine roots. *J Nanopart Res* 17:448. doi:[10.1007/s11051-015-3246-4](https://doi.org/10.1007/s11051-015-3246-4)
- Syu YY, Hung JH, Chen JC, Chuang HW (2014) Impacts of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression. *Plant Physiol Biochem* 83:57–64

- Tawaraya K (2003) Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Sci Plant Nutr* 49:655–668
- Tripathi DK, Shweta SS, Swati S, Pandey R, Singh VP, Prasad SM, Dubey NK, Chauhan DK (2017) An overview on manufactured nanoparticles in plants: uptake, translocation, accumulation and phytotoxicity. *Plant Physiol Biochem* 110:2–12
- Wang F, Liu X, Shi Z, Tong R, Adams CA, Shi X (2016) Arbuscular mycorrhizae alleviate negative effects of zinc oxide nanoparticle and zinc accumulation in maize plants – a soil microcosm experiment. *Chemosphere* 147:88–97
- Yin L, Colman BP, McGill BM, Wright JP, Bernhardt ES (2012) Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. *PLoS ONE* 7:1–7

Index

A

- Abiotic stresses, 46, 48, 57–65, 73, 76, 79, 108, 198, 223–228, 234, 240, 248, 284, 289, 294
- Abscisic acid (ABA), 61, 195
transporter, 123
- Acacia nilotica*, 74
- Acid phosphatase, 23, 75, 227
- Acinetobacter lwoffii*, 29
- Activated carbon, 319
- Agrobacterium*, 213
- Agrochemicals, 87, 90, 313
- Agro-ecology, 59
- Agroecosystems, 89, 90, 136, 144–145, 209, 212
- Agroforestry, 211, 219
- Ajmalicine, 291
- Alkaline phosphatases, 75, 227, 240
- Alkaloids, 216, 259, 260, 264, 265, 284, 285, 288, 291, 293, 294
- Allium sativum*, 73
- Aloe vera*, 248, 252–256, 262–263, 268, 290
- Aloin, 262–263, 268, 290
- Amanita muscaria*, 138, 185, 194, 197
- Amaranthaceae, 216
- AMF. *See* Arbuscular mycorrhizal fungi (AMF)
- AMF-based technology, 90–94
- Amino acid metabolism, 240
- 1-Aminocyclopropane-1-carboxylate (ACC)
deaminase, 28
- Anadenanthera colubrina*, 287
- Anthocyanin, 288
- Anthraquinones, 268, 285
- Antibiotics, 45
- Antifungal activity, 274
- Anti-malarial drug, 274
- Antimicrobial agents, 284, 304
- Antioxidants
defense, 61, 73, 78, 121, 227
enzymes, 77, 78, 80, 225
machinery, 108
signaling, 224
- Antioxidative defense mechanism, 77–79
- Apoplast, 161, 190, 196, 197
- Appressoria, 192
- APX. *See* Ascorbate peroxidase (APX)
- Aquaporins, 23, 38, 61, 111, 125, 166, 167, 317, 318
- Arabidopsis thaliana*, 195
- Arachis hypogea*, 80
- Arbuscular mycorrhizal fungi (AMF), 13, 43–51, 58, 71–81, 88–97, 107–124, 145, 151, 167, 170, 171, 187–189, 198, 200, 209, 211–216, 218, 224–226, 228, 234, 240, 274, 275, 285–289, 292–294, 307, 311–315, 318–320
inoculants/inoculation, 75–77, 89, 91, 117, 119, 121, 211, 214, 218
- Arbuscular mycorrhiza/mycorrhizal (AM), 3, 8, 9, 36, 87–98, 110, 186–188, 198, 199, 211, 234, 287–289, 312, 314–316
symbiosis, 2, 3, 11, 95, 149, 161, 164, 165, 185, 187, 188, 191, 193, 194, 197, 210, 211, 286
- Arbuscules, 47, 61, 109, 116, 150, 160, 185, 187, 190, 192, 193, 197, 210, 251, 312, 314, 315
- Arbutoid mycorrhizas, 194
- Argania spinosa*, 218

- Arginine, 140
Aristolochia elegans, 290
 Arsenate reductase (ACR), 121, 124
 Arsenate toxicity, 111
 Arsenic, 107–124
 menace, 108–110
 uptake, 110–111
Artemisia annua L., 274, 287, 290, 293
 Artemisinin, 273–279, 287, 290, 293
Arthrobacter sp., 29–31
 Arum-type, 160, 166
 Ascocarps, 23, 24, 27, 34–36
 enzymes, 23, 24, 27, 34–36
 Ascorbate, 110, 123
 Ascorbate peroxidase (APX), 114, 123, 224,
 226, 227
 Aseptate, 189
Asparagus racemosus, 252, 255, 266–267
 Asteraceae, 274
 Auto-fluorescent, 252
 Auxins, 28, 195, 268
 Auxin transport, 195
 Azospirillum, 94, 291, 293
 Azotobacter chroococcum, 94, 135, 136, 138,
 227, 267, 273–280
- B**
Bacillus
 B. atropheus, 29
 B. coagulans, 292
 B. megaterium, 29, 291, 293
 B. simplex, 29
Bacopa monnieri, 248, 253, 255, 256, 290, 291,
 317
 Bacterial community, 320
 Basidiomycetes, 44, 48, 314
Basidiomycota, 189, 194, 311
 Batch fermentations, 307
 Berberine, 288
 β -cyclodextrin, 23
 Biocontrol of nematodes, 45, 46, 49–51
 Biodegradable, 235, 304
 Bio-fertilizers, 88, 144, 145, 188, 247, 252,
 279, 289, 307, 313
 Biological control, 47
 agents, 45, 252
 Biological diversity, 210, 212
 Biological hardening agent, 289
 Biological remediation, 76
 Biomedical imaging, 304
 Bio-methylation, 118, 120
 Biopesticides, 88
 Bio protectant, 289
 Bioprotectors, 188
 Bioreactor, 31, 33, 303–309
 Bioremediation, 88, 200
 Biotic stresses, 44, 49, 51, 58, 62–65, 74, 135,
 224, 227, 234
 Biotransformation, 110, 118–119
 Biotroph/biotrophic, 14, 45, 48, 49, 198, 226,
 234
 pathogens, 45
 phase, 50
Blumeria graminis, 49
 Brassicaceae, 216
Brassica napus, 145
- C**
¹⁴C, 198
Caenorhabditis elegans, 119
Cajanus cajan, 74, 76, 80
 Calcium spiking, 314
 Calvin-melvin cycle, 112, 122
 Camptothecin, 287
 Carbon cycling, 188
 Carbonic anhydrase, 72
 Carbon nanotubes (CNTs), 318
 Catalase (CAT), 74, 77–80, 110, 114, 123, 224,
 226
 Catecholase, 35
 Catharanthine, 258–260
Catharanthus roseus, 253, 255, 287, 291
 C efflux, 12
 Cell death, 51, 59, 113, 226, 248
 Cell toxicity, 58, 59
Centella asiatica, 290
 Chelation, 121, 123, 198, 200
 Chemical fumigants, 45
 Chemotaxis, 48
 Chenopodiaceae, 216
 Chitinases, 49
 Chlamydospores, 50, 92, 141, 239, 252, 308
 Chlorophyll, 28, 74, 76, 77, 110, 112, 122, 240,
 267, 273, 276–277, 317
 Chlorophyllase, 76
 Chlorophyll meter, 28
 Chlorosis, 112
Choironomyces magnusii, 26
Cicer arietinum L., 76, 167, 292, 317
Citrus
 C. reticulata, 225
 C. sinensis, 4, 5, 74
 Cluster analysis, 158
 Coenocytic, 153, 155, 156, 160, 190
¹⁴CO₂ labeling, 3
Coleus forskohlii, 248, 252, 253, 255–258, 287,
 290
Collimonas pratensis, 146

Colonization, 11, 38, 46–51, 61, 63, 75, 77, 78, 80, 88, 91, 92, 94–96, 115–122, 150, 151, 158–162, 164, 165, 169, 187, 189–193, 195–200, 212, 218, 228, 234, 237–239, 268, 275, 276, 278, 286, 312, 314–316, 319, 320

Combating oxidative stress, 110

Coumarins, 287

¹¹C-positron tomography, 15

Cresolase, 35

Crocus sativus, 49

Cropping systems, 59, 90–93, 216, 217

Crop productivity, 72, 313

Crop protection, 45, 89

Crop rotations, 45, 91, 92

Crotalaria juncea, 216, 217

Cryptic, 24, 26

Cucurbita pepo, 74

Cultural management, 90–91

Curcuma longa L., 290, 293

Curcumin, 290, 293

Cyamopsis tetragonoloba, 317

Cyclodextrins (CDs), 32

Cycloheximide, 143

Cyclophilin A, 252

Cymbopogon citratus, 286, 287

Cysteine, 121, 123

Cyst nematodes, 44, 49, 50

Cytokinins, 193, 195

D

Dark septate endophytes (DSE), 289, 290

Desertification, 59, 60

Desert truffles, 23–39

Detoxification, 78, 79, 120, 121, 123, 224

Dianthus caryophyllus, 63

Dicotyledonous, 88

Dilution effect, 110, 117

Diosgenin, 291

Disease resistance, 210, 250, 279, 313

DNA fingerprinting, 97

DNA markers, 96, 97

DNA polymerase, 153

DNA polymorphism, 152

Dose-dependent manner, 319

Drought

- resistance, 88
- stress, 38, 60–61, 79, 184, 188, 199, 225, 251
- tolerance, 38, 97, 211, 225, 250

Drought-prone condition, 89

Drug delivery, 304

E

ECM. *See* Ectomycorrhizae (ECM)

Ecological aspects, 188–189

Ecological niches, 47

Ecological sustainability, 217

Ectendomycorrhizas, 34, 189, 234

Ectomycorrhizae (ECM), 11, 185, 186, 189, 194–197, 234, 313

Ectomycorrhizal fungi (EMF), 31, 188, 194–197, 312, 313

Ectotrophic interactions, 188

Elicitation, 268, 284, 292

Elicitor, 284

Endodermis, 47, 196, 252

Endogonaceae, 314

Endogone arenacea, 6

Endomycorrhizal fungi, 313, 314

Endomycorrhizas, 34, 187–189, 194, 234

Endoparasitic, 44, 46, 47

Endophyte, 49, 136, 184, 189, 197, 234, 235, 268, 286, 289, 290, 293, 294

Endophytic fungi, 49, 249, 290

Endosphere, 137

Environmental pollution, 89

Epimedium wushanense, 290

Epiphyte, 136

Ericoid mycorrhizal, 11

Essential oils, 284–291, 293, 294

Ethylene, 235, 252

Eugenol, 260–261, 288, 291

External hyphae (ERM), 88, 93, 194

Extracellular metabolites, 31

Extraradical, 109, 120, 151, 158, 159, 168, 192

Extraradical mycelium (ERM), 8, 88, 120, 150, 160, 162, 169, 190, 196, 197, 199

Extra radical network, 63

F

Flavobacterium sp., 29–31

Flavonoids, 268, 285, 287–293

Flavonols, 268

Fodder grasses, 92

Food chains, 13–14

Food quality, 89

Forskolin, 257–258, 287

Fructification, 36

Functional diversity, 137, 149–171, 188

Fungal communities, 2, 6, 7, 152

Fungal inocula, 212

Fungal inoculum, 32, 60, 166

Fungal metabolites, 49

Fungal pathogens, 35, 47, 49, 144, 198, 252, 289
 Fungal propagules, 209, 211
 Fungal sheath, 196, 197
Funneliformis
 F. caledonium, 6
 F. mosseae, 4, 7–9, 47, 121, 158, 167, 289, 319
Fusarium culmorum, 49

G
 Gel analysis, 142
 Gelatinous, 44
 Genetic diversity, 97, 135, 149, 151–157, 160, 165, 171
 Genetic manipulation, 94
 Genetic variation, 97, 151, 153, 156, 157, 165, 171
Geosiphon
 G. pyriforme, 185
 G. pyriformis, 12
Gigaspora margarita, 5, 7, 116, 124, 141, 158, 162, 288
 Glomales, 187
 Glomalin, 14, 61, 63, 88, 110, 120, 198
 Glomeromycetes, 47, 149, 211
Glomeromycota, 12, 73, 149, 151, 157, 163, 185, 189, 311, 312, 314
Glomus
 G. aggregatum, 118, 293
 G. claroideum, 7, 162, 164
 G. etunicatum, 73, 153, 154, 156, 161, 288
 G. fasciculatum, 5, 6, 9, 73, 274, 287, 292, 293
 G. intraradices, 4–7, 60, 61, 118, 152, 158, 161, 163–170, 189, 216, 287, 292
 G. macrocarpum, 274, 287
 G. mosseae, 4, 7, 9, 73, 116, 158, 161, 164, 166, 168, 170, 199, 226, 286, 287
Gluconacetobacter, 138
 Glucose transporters, 139
 Glutamate dehydrogenase, 142, 143
 Glutathione (GSH), 114, 121–123, 224
 Glutathione-ascorbate cycle, 227
 Glutathione reductase (GR), 78, 224
 Glutathionylation, 122
 Glycine betaine, 71, 80, 110, 123
Glycine max, 5, 9, 50, 74, 76, 79, 291
 Glycolytic pathway, 139
 Glycoproteins, 63, 88, 120, 268
Glycyrrhiza glabra, 248, 252, 255, 256
 Glycyrrhizin, 287

Goodyera repens, 10, 11
 Gramineae, 95
 Groundwater, 59, 109
 Growth response, 71–81, 141, 161–164, 169, 218
 GSH. *See* Glutathione (GSH)
 GTP-binding protein, 140
 Guaiacol peroxidase (GPX), 224, 226

H

Hartig net, 194, 196, 197, 315
 Hatching, 48
 Haustorial-plant interface, 198
 Haustorium, 192
 Heavy metals stress, 199–200
Helianthus annuus, 118, 319
Helicotylenchus spp., 46
 Herbicide, 108, 226
 Hexose transporter, 185, 197
Holcus lanatus, 119
 Holobiont, 136, 145, 216
 Homokaryosis, 153, 155, 156
 Homokaryotic, 153, 154, 189
Hordeum vulgare, 96, 115
 Hydrolytic enzymes, 195
 Hydroperoxides, 35, 36
 Hydroponic, 113, 268
 Hyoscyne, 286
 Hyoscyamine, 291
Hyoscyamus niger, 291, 294
 Hypaphorine, 195
 Hyperaccumulator plants, 113, 124
 Hyperaccumulators, 113, 123–124
 Hypericin, 287
 Hyperosmotic stress, 75
 Hypersymbiotic, 13
 Hypertrophy, 192
 Hyphopodium, 150, 190, 314, 315
 Hypogeous, 24, 26
 Hypothecium, 35

I

Indole acetic acid, 145
 Indole glucosinolates, 50
 Induced systemic resistance, 49, 51
 Inoculum, 27, 30–32, 80, 89, 90, 92–94, 159, 167, 168, 212, 213, 217, 234, 235, 248, 252, 305–307
 production, 30–32, 90, 92–94
 Insect pathogen, 63, 64
 Intraradical hyphae, 150

- Intraradical mycelium (IRM), 9, 150, 161, 190, 196
 Invasion, 47, 64, 312
 Invertase(s), 12, 51, 197
 Invertase genes, 197
 Ion toxicity, 72
 IRM. *See* Intraradical mycelium (IRM)
 Iron oxide nanoparticles, 319
 Isoflavone, 291
 Isotopic C labeling, 3
 Isotopic pulse-chase labeling, 10
 ITS-rDNA sequence, 24
- J**
 Jasmonic acid, 193
 Jatrorrhizine, 288
- K**
 Keystone organisms, 150
 K-strategists, 168, 169
- L**
Laccaria bicolor, 194, 195
 Lactophenol cotton blue, 50
Lactuca sativa, 74, 292
Lens culinaris, 112
 Lichens, 184
 Linoleic acid, 35
Linum
 L. album, 290
 L. usitatissimum, 115, 161
 Lipid peroxidation, 35, 113, 123
 Lipid radical production, 113
 Lipochitoooligosaccharide, 191
 Lipopolysaccharides, 268
 Lipoxigenases (LOXs), 34–36
 Liquid fermentation, 31
Lotus
 L. glaber, 77, 79, 80
 L. japonicas, 95, 193
 Low-density polyethylene (LDPE), 93
 Luxury goods, 170
Lycopersicon esculentum, 115, 161
Lycopersicum esculentum, 235–239
- M**
 Macroconidia, 49
 Macronutrients, 61, 88, 239, 240, 294
Magnaporthe oryzae, 97
 Malonaldehyde, 72
 Mantle, 194, 196, 197, 314–315
 Marker assisted selection (MAS), 97
Mattiroloomyces terfezioides, 26
Medicago truncatula, 7, 8, 62, 115–117, 119, 121, 161, 162, 166, 169, 185, 225, 226
Melastoma malabathricum, 116, 117
Meloidogyne
 M. incognita, 46, 63, 225
 M. javanica, 225
 Menthone, 291
 Metalloids (As), 107, 114
 Metallothioneins (MTs), 108, 120, 121
 Metallothionens, 200
 Metal toxicity, 88, 116, 199
Microbacterium paraoxydans, 29
 Microbe-associated molecular patterns (MAMP), 47
 Microbe-supportive, 90
 Microbe-targeted Approach, 212–216
 Microbial activity, 120, 218
 Microbial community(ies), 89, 137, 144, 146, 216, 285, 318, 320
 Microbial disturbances, 89
 Microbial incubator, 136–137
 Microbial symbiosis, 240, 283–295
 Microbiome, 48, 136, 143–146
 Microbiota, 46, 58, 136, 137, 143–146, 211, 215, 218, 274
 Microcosms, 164, 170
 Micronutrients, 61, 74, 80, 88, 234
 microRNA-based techniques, 15
 Microtubules, 192
 Mineralization, 234
 Molecular dialogue, 215
 Monocotyledonous, 88, 267
 Monocropping, 92
 Monodehydroascorbate reductase (MDHAR), 224
 Monosaccharide transporters (MSTs), 12
 Monotropoideae, 14
 Morphospecies, 163
 Mucilage, 137
 Multigenomic, 151
 Multi-walled carbon nanotubes (MWCNTs), 318
 Mycelium, 7–11, 27, 30–32, 34, 50, 60, 62, 115, 118, 120, 140, 155–157, 162, 190, 240, 308, 314
 Myc factors, 191
 Mycoheterotrophic, 11
 Mycoheterotrophic plants, 2, 13, 14, 188

- Mycorrhiza, 2, 3, 10, 14, 24, 28, 33, 36, 44, 47,
 59–64, 94, 96, 98, 110, 118, 120, 123,
 124, 160, 184–185, 187, 225, 234, 242,
 274, 289, 313–315, 318–320
 Mycorrhizae, 189, 237, 313
 Mycorrhizal associations, 2, 28, 78, 158, 171,
 184, 225, 240, 313
 Mycorrhizal colonization, 78, 116, 162,
 198–200, 237–239, 319
 Mycorrhizal fungi, 2, 13, 24, 30, 43–51, 57–65,
 73, 89, 96, 138, 184, 185, 188, 189, 194,
 197, 199, 200, 210, 215, 217, 237, 240,
 286, 293, 313, 320, 321
 Mycorrhizal inoculation, 61, 73, 119, 122, 199,
 294
 Mycorrhizal interface, 163, 192–197
 Mycorrhizal networks, 160
 Mycorrhizal pathway (MP), 161–163
 Mycorrhizal responsiveness (MR), 94, 96
 Mycorrhizal roots, 28, 109, 159, 160, 165, 193,
 197
 Mycorrhizal symbiosis, 1, 2, 11, 26, 36, 59–61,
 65, 183–201, 212, 215, 250–256
 Mycorrhizal uptake pathway, 109, 115
 Mycorrhiza/mycorrhization helper bacteria
 (MHB), 28, 33, 215
 Mycorrhization, 27, 28, 31, 33, 36, 73, 80, 95,
 185, 250, 251, 286
 Mycorrhizosphere, 63, 89, 121, 215
 Mycorrhizosphere soil, 28
 Mycotrophy/mycotrophic, 14, 164, 189, 213,
 216
- N**
 Nanoclays, 312
 Nanoembedded, 305, 306
 Nano-fertilizers, 315
 Nano-herbicides, 315
 Nanomaterials, 303–309, 312, 315
 Nanoparticle interaction, 318–320
 Nanoparticles, 303, 304, 308, 312, 315–321
 Nano-pesticides, 315
 Near-isogenic lines (NILs), 96
 Necrosis, 59, 248
 Necrotrophic, 45, 48, 49
 Nematicides, 45
 Nematodes, 44–51, 216, 225, 227
 Niche, 58, 90, 136–137, 141, 144, 146, 185
Nicotiana tabacum, 225
 Nitrate reductase, 72
 Nitrification, 145
 Nitrogen cycling, 285
 Nitrogen fixation, 164–165, 224, 274, 276, 290
 Nitrogen-fixing bacteria, 63, 275, 279
 Nitrogen-fixing legume, 216
 Nod factors, 191
 Nodulation, 95, 164–165, 191, 292
Nostoc punctiforme, 12
Novosphingobium panipatense, 29
 Nutrient(s)
 acquisition, 44, 94, 151, 279, 285, 319
 availability, 13, 251
 cycling, 75, 188, 212
 profile, 75
 stress, 61–62
 uptake, 44, 46, 48, 50, 51, 61, 73–76,
 161–164, 167, 168, 171, 211, 234, 242,
 251, 268, 274, 286, 289, 294, 319
- O**
 Obligate biotrophs, 189, 190
 Obligate symbionts, 73, 150, 187, 188, 314
Ocimum sp., 248, 252
 O. basilicum, 80, 288, 291
 Off-season tillage (OST), 91
 Oligosaccharides, 32
 Oomycetes, 62
 Ordovician, 189
 Organic acids, 13, 120, 137, 293
 Organic manure, 239
 Organic nutrients, 195, 234
 Orthophosphate, 109, 114, 115, 240
Oryza sativa, 96
 Osmolytes, 61, 72–74, 77, 79, 80
 Osmolytes enzymes, 77
 Osmoregulation, 80
 Osmotic potential, 199, 249
 Osmotic stress, 72
 Outbreeding depression, 218
 Oxidative damage, 61, 72, 77, 78, 112–114,
 122–123
 Oxylipins, 35, 36
- P**
Paenibacillus lentimorbus, 292
 Palmatine, 288
 Parasitoids, 64
 Parthenogenesis, 44
 Pathogen attacks, 284

- Pathogenic bacteria, 62
 Pathogenic fungi, 62, 195
 PCR-RFLP, 158
Pelargonium graveolens, 292, 295
Pennisetum glaucum, 75, 78
 Periarbuscular membrane, 185, 190
 Periarbuscular space, 119, 193
 Peridium, 26, 28, 35
 Perlite, 28, 30, 213, 214
 Peroxidase (POD), 77, 78, 80, 123, 224, 226, 227
 Pesticides, 62, 89, 108, 233, 284
 PGPRs. *See* Plant growth-promoting rhizobacteria (PGPRs)
Phaseolus vulgaris, 226
 Phenolics, 34, 48, 137, 268, 284, 285, 287, 289, 292, 293
 Phosphatases, 34, 227, 240, 293
 Phosphate
 acquisition, 210
 depletion zone, 88
 fertilizers, 60
 solubilization, 28–30, 138, 290
 solubilizing, 63, 274
 transporters, 95, 118, 184, 197
 Phosphate-solubilizing bacteria (PSB), 63, 94
 Phosphodiesterase, 240
 Phosphomonoesterase, 240
 Phosphorus (P), 1, 48, 61, 62, 75, 87–98, 150, 157, 160, 164, 165, 167–170, 187, 197–198, 210, 211, 225, 241, 252, 276, 290, 292–294, 312, 314
 uptake, 62, 75, 92, 93, 95, 98, 113–118, 151, 158, 161–163, 197–198, 241, 314
 Photoassimilation, 13
 Photocatalysis, 304
 Photomixotrophic, 27
 Photosynthate, 5, 10, 137, 150, 188, 196, 198
 Photosynthesis, 1–3, 7, 10, 38, 72, 76, 77, 122, 164, 249, 251, 277, 286
Phragmites australis, 74, 80
Phyllobacterium bourgognense, 29
 Phyllospheric, 136
 Phylogenetic analysis, 25, 28
Phytophthora parasitica, 63
 Phytochelatins (PCs), 108, 114, 120–123, 200
 Phytochemicals, 285, 293, 294
 Phytohormone, 48–49, 274, 279
 Phytopathogenic fungi, 197
 Phytophagous insects, 63–64
 Phytoremediation, 60, 124, 188
 Phytotoxicity, 318
Picoa
 P. juniper, 26
 P. lefebvrei, 26, 27, 31, 36
Piper nigrum, 74
 PiPT. *See* Pi transporter (PiPT)
Piriformospora
 P. indica, 43–51, 135–146, 185, 225–228, 233–242, 248, 251–256, 267, 268, 273–280, 286, 289, 290, 292–294, 303–309
 P. williamsii, 289
Pisolithus tinctorius, 194, 196
 Pi transporter (PiPT), 95, 114–116, 119, 166, 185, 240, 252
Pityrogramma calomelanos, 116
 Plant community(ies), 2, 64, 143, 184, 200, 211
 Plant disease, 234, 313
 Plant-Fungus Communication, 184–185
 Plant growth-promoting rhizobacteria (PGPRs), 28, 33, 63, 94, 138, 215–217, 267, 274, 275, 290–294
 Plant health, 59, 77, 292
 Plant hormones, 48, 251
 Plant-microbe association, 135, 137
 Plant-microbe interaction, 224, 225, 234, 249–250, 252
 Plant-microbial association, 224
 Plant-microbial community, 146
 Plant-mycorrhiza interaction, 227, 228
 Plant-mycorrhizal association, 223–228
 Plant pathogens, 45, 235, 313
 Plant production, 27–30, 32–33, 144
 Plant productivity/yield, 58, 59, 136, 144, 146, 164, 212, 225, 267, 279
 Plant tolerance, 59, 60, 62, 74, 76, 199, 268
 Plasmolysis, 72
 POD. *See* Peroxidase (POD)
 Polyamine, 79
 Polyethylene glycol, 34
 Polymorphism, 97, 152, 153, 157, 189
 Polyphosphate, 115, 169, 200
Pratylenchus zeae, 46
 Predators, 64
 Prepenetration apparatus (PPA), 314
 Proline, 72, 74, 79–81, 114, 123
Prosopis juliflora, 251
 Proteins, 72, 75, 79, 80, 111, 112, 114, 118, 119, 121–123, 138–144, 193, 195, 303, 317
 Protochlorophyllide reductase, 112

- Proton-ATPase activity, 12
Pseudohypericin, 287
Pseudomonas
P. aeruginosa, 143
P. brenneri, 30, 31
P. fluorescens, 267
P. fluorescens, 28, 29, 31, 33, 94, 138, 291, 293, 294
P. putida, 138, 291, 293, 294
P. striata, 292
Pteris ensiformis, 114
Pure culture, 116, 226, 307
- Q**
Quantitative trait loci (QTLs), 96, 97
Quorum sensing, 290
- R**
Radioisotope labeling, 162
Rancidity, 35
Reactive oxygen species (ROS)
generation, 77, 224, 226, 227
metabolism, 223–228
signaling, 226, 228
Recalcitrant soil organic matter, 14
Redox homeostasis, 224
Reporter genes, 15
Restoration, 250, 286, 312
Rhizobial symbiosis, 191, 226
Rhizobium-legume symbiosis, 198
Rhizobium radiobacter, 29
Rhizobium strains, 95, 164
Rhizoctonia solani, 63
Rhizomorphs, 196
Rhizophagous insects, 64
Rhizophagus
R. clarus, 7, 9, 158, 163, 214, 287, 288
R. fasciculatus, 5, 9, 289
R. intraradices, 4–8, 117, 118, 121, 123, 163, 287
R. irregularis, 8, 152, 156, 167, 214, 216, 289
Rhizoplane, 137, 290
Rhizosphere, 12, 28, 48, 58, 114, 115, 118, 120, 121, 136–138, 143–146, 184, 195, 196, 199, 200, 224, 234, 240, 248, 251, 252, 274, 285, 290, 292, 294, 312, 320
Rhizospheric, 80, 136, 143, 146, 275, 319
Rhizospheric regions, 138, 249, 285, 312
Rhodococcus sp., 30
Ribosomal RNA (rRNA) sequences, 155, 189
Rice-based cropping, 90–93
Root architecture, 80
Root cortex, 50, 109, 150, 186, 188, 196, 314
Root endophytic fungus, 145, 286–290
Root exudates, 5, 13, 46, 48, 137, 159, 190, 195, 285
Root exudation, 250, 251
Root infection, 63
Root-knot nematodes (RKN), 44, 46
Root tip, 195, 315
ROS. *See* Reactive oxygen species (ROS)
Rotation options, 91–92
Rotylenchulus reniformis, 46
r-strategists, 168, 169
Rubisco, 112
- S**
Salicylic acid-mediated, 50
Salinity
stress, 72–81, 199, 227, 252, 267, 268, 294
tolerance, 71–81
Salt tolerant crops, 59
Salvia officinalis, 286, 289, 291, 294
Saponins, 143, 285, 293
Saprotrophes, 14
Scarification, 27
Scopolamine, 291
Scutellospora
S. calospora, 6, 158, 166, 168, 288
S. pellucida, 153, 158
Sebacinaceae, 251
Sebacinales, 44, 48, 289
Sebacina spp., 44
Secondary metabolites, 31, 34, 49, 143, 144, 257–268, 279, 284–293
Seed germinations, 27, 33, 316–318
Septate, 189, 314
Sequestration, 108, 117, 120, 123, 124, 200
Serratia plymuthica, 143
Sesbania
S. aegyptiaca, 74, 77
S. grandiflora, 74, 77
Shatavarin, 266, 267
Siderophores, 28–30, 200
Silver nanoparticles, 317
Simple sequence repeats (SSRs), 96, 97
Sinorhizobium meliloti, 29, 291
SOD. *See* Super-oxide dismutase (SOD)
Soil aeration, 72
Soil biota, 13, 14, 59
Soil-borne diseases, 62, 88
Soilborne microsymbionts, 274

- Soil-borne pathogens, 45, 313
 Soil debris, 26
 Soil disturbance-induced (SDI), 91–92
 Soil fungi, 1, 73, 92, 187, 251
 Soil microbial community, 89, 285
 Soil microbiota, 58, 211, 218
 Soil phosphate, 251, 286
 Soil porosity, 72
 Soil resilience, 59
 Soil-root interface, 161
 Soil salinity, 59, 60, 71–72, 77, 248, 249, 267
 Soil salinization, 58, 72, 248
 Soil solarization, 93
Solanum viarum, 292, 293
 Soybean, 45, 50–51, 78, 123, 164, 225, 234, 240, 241, 317, 320
 Spore density, 121
Stenotrophomonas rhizophila, 29
Stevia rebaudiana, 289, 292, 293
 Steviol glycoside, 289, 292, 293
 Stevioside, 292, 293
 Stomatal movement, 249
 Stress tolerance, 48, 49, 51, 58, 79, 108, 199, 226–228, 252
 Strigolactones, 48, 190
 Stromules, 192
 Sucrose
 synthases, 51
 transporters, 12
 Sugar fluxes, 12
 Sulfate transporters, 193
 Super-oxide dismutase (SOD), 77, 78, 80, 114, 123, 224, 226, 319
 Superoxide radicals, 113, 225
 Sustainability, 38, 90, 219, 225
 Sustainable agriculture, 88, 136, 144, 145, 188, 233
 Sustainable efforts, 58
 Sustainable productivity, 88
 SWEET translocator, 12
 Sylviculture, 36–39
 Symbionts, 11, 14, 45, 48, 58, 150, 169–171, 187, 195, 199, 200, 214, 252, 289, 294, 311, 313–315
 Symbiosis, 2, 3, 10, 11, 28, 34, 38, 39, 48, 58, 60, 65, 77, 79, 88, 91, 94, 95, 149–152, 157, 158, 161, 164–166, 169, 170, 184, 185, 187, 188, 191–197, 210, 211, 224, 286, 314, 318
 Symbiosome, 197
 Symbiotic associations, 44, 48, 73, 138, 200, 226, 240, 250, 251, 274, 285, 292, 313
 Symbiotic efficiency, 164, 171, 218
 Symbiotic relationship, 73, 227, 286, 313, 315
 Syncytia, 47, 51
 Syncytium, 44
 Synthetic agrochemicals, 90
 Systemic resistance, 46, 48, 49, 51
- T**
Tagetes erecta, 116
 Tanshinone, 291
 TBA. *See* Thiobarbituric acid (TBA)
Terfezia
 T. arenaria, 26, 27, 36
 T. boudieri, 26, 27, 36, 38
 T. canariensis, 24, 26
 T. claveryi, 24, 26–28, 31, 32, 34–38
 T. fanfani, 26
 T. leptoderma, 26
 Terpenoids, 284
 Thiobarbituric acid (TBA), 114, 122–123
 Thylakoid membrane, 112
 Thymol, 288, 293
Thymus daenensis, 293
 Tillage, 90–92, 212
Tirmania
 T. nivea, 26, 27, 36, 38
 T. pinoyi, 26
 Tissue culture raised plants, 249, 253, 254
 Toxicity, 49, 58, 107–124, 143, 199, 304, 315, 318
 Transcriptional analysis, 166
 Transpiration, 60, 249
 Transporter genes, 95–96, 166
 Trehalose, 13, 165
Trichoderma, 289
 T. harzianum, 292, 293
Trifolium alexandrinum, 72, 74
 Tripartite symbiosis, 165
Triticum aestivum, 96, 115
 Tryptophan, 195
Tuber
 T. lacunosum, 26
 T. oligospermum, 26
 Tylenchid nematodes, 44
 Type III secretion protein, 143
 Tyrosinase, 34, 35
- U**
 Upland rice, 87–98
 Urease, 139, 140, 240

V

Variovorax paradoxus, 29, 30
Vermicompost, 233–242
Vermiculite, 213, 306
Vesicles, 116, 120, 140, 141, 150, 159, 169,
187, 190, 192, 312, 314
Vicia faba, 4, 9
Vinblastine, 258–260, 287
Vinca rosea, 248, 258–260
Vincristine, 258–260
Vindoline, 258–260
Vitamins, 35, 215, 251, 307

W

Water stress, 34, 38, 44, 59, 60, 74, 88, 210,
294
Withaferin A, 264–266, 290

Withania somnifera, 235, 248, 252, 253, 255,
256, 264–266, 290, 291
Withanolide A, 264–266

X

Xylose, 193

Z

Zea mays, 61, 74, 96, 225
Zeolites, 312
Zinc oxide nanoparticles
(ZnONPs), 317, 319
Zizyphus nummularia, 251
Zn uptake, 46
Zygomycota, 187